EVOLUTIONARY DYNAMICS OF *Pinus taeda* L. IN THE LATE QUATERNARY: AN INTERDISCIPLINARY APPROACH

A Dissertation

by

MOH'D ALI AL-RABAB'AH

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2003

Major Subject: Forestry

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Approved as to style and content by:

Claire G. Williams (Co-Chair of Committee)	Raghavan Srinivasan (Co-Chair of Committee)
Fred Smeins (Member)	Ben Wu (Member)
C. T. Smith (Head of Department)	

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ABSTRACT

Evolutionary Dynamics of *Pinus taeda* L. in the Late Quaternary: An Interdisciplinary Approach. (August 2003)

Moh'd Ali Al-Rabab'ah, B.S., University of Jordan;

M.S., University of Jordan

Co-Chairs of Advisory Committee: Dr. Claire G. Williams
Dr. Raghavan Srinivasan

Pinus taeda L. population dynamics, migration patterns and genetic structure were investigated over geological time scale (the past 21,000 years), historical time scale (the past 500 years) and recent time scale (the past 50 years ago) using multi-source data and an interdisciplinary approach. Population genetics, microsatellite DNA markers, DNA fingerprinting techniques, fossil records, geological history, historical records, aerial photographs, soil maps, weather data, remote sensing and geographic information systems (GIS) were used to assess the dynamics of *P. taeda* populations especially for the Lost Pines (LP), a disjunct population at the westernmost edge of the species range.

Pinus taeda populations east and west of the Mississippi River Valley are genetically differentiated. Eastern populations had higher allelic diversity and diagnostic alleles than western populations. Gene flow estimates are high. Allelic diversity patterns and the distribution of diagnostic alleles are attributed

to the prevailing wind direction. Differentiation east and west of the MRV was attributed to separation to two refugia during the Pleistocene.

The Lost Pines population is believed to have undergone one or more bottleneck events with the apparent loss of rare alleles. Despite the bottleneck, allelic richness was similar for the LP and the control population from the Western Gulf (WG) population. Population size contraction and expansion of the LP was attributed to climate change in central Texas over geological time scale.

The natural origin of the Lost Pines was investigated. Multivariate and clustering techniques and assignment and exclusion methods using DNA markers show that the LP population shared ancestry with the WG populations with no evidence for admixture from other sources. Historical records parallel this conclusion.

With the absence of logging within Bastrop and Buescher State Parks, *P. taeda* area and patch size increased from 1949 to 1995. Thirty six percent of the pine patches observed in 1949 had disappeared by 1995 by merging. Landscape pattern analysis shows significant dynamics. The distribution of *P. taeda* in Bastrop County was associated with sandy light topsoils, clayey heavy sub-soils and high water permeable soils. *Pinus taeda* grow on various soil types as

well. Growing on these soils under current climatic conditions may compensate for the precipitation regime in this area.

DEDICATION

I dedicate this dissertation to almighty ALLAH (الله سبحانه و تعالى) for his great mercy bestowed on me and my family throughout my life and to His Messenger Prophet MOHAMMAD (محمد صلى الله عليه وسلم) the one who came with the guidance for the whole universe.

I also dedicate this dissertation to my parents who strived to put me on the right path and for their support and prayers throughout the years of my life, to my wife who supported me throughout this lengthy journey with her compassion and love, to my father in law (may ALLAH have mercy on him), and to my mother in law for her wise advice and prayers.

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Second, special thanks are extended to the colleagues from Dr. Williams' lab especially Lisa Auckland for her hard work developing and running all the samples and markers used in this study. Others from the same lab are Thomas Bui, Zhou Yi, David Gwaze, Humberto Reyes-Valdes, Virginia Minihan and Ann Scarpa. Thanks to the staff at the Spatial Science Laboratory for their technical help as well. Thanks to Dr. Thomas Byram for securing germplasm.

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Of course, I will not forget my wife and our two beautiful daughters and son. I certainly deprived them of some of their precious time because of my study and long nights in the lab and office. I will try hard to give some back inshallah and compensate for all this time.

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CHAPTER I

INTRODUCTION

Understanding migration patterns and natural history of *Pinus taeda* L. (loblolly pine) in response to changing environmental conditions on geological, historical and recent time scales is the critical element in predicting the future response of this species to changing environmental conditions in the future. This assertion is true for plant species worldwide (Pitelka and The Plant Migration Workshop Group, 1997; Higgins and Richardson, 1999) and especially critical for forest species in the United States (Roberts, 1989).

The rate and magnitude of climatic changes anticipated for the coming century are unprecedented, presenting unique challenges to the biota of the planet (Jackson and Overpeck, 2000). Responses of terrestrial plant populations to changing environmental conditions range from tolerance *in situ* to moderate shifts in habitat to migration through seed dispersal and/or extinction, depending on magnitudes and rates of environmental change (Jackson and Overpeck, 2000). The ability to predict migration and colonization rates is needed for predicting how native species will distribute themselves in response

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This dissertation follows the style and format of the journal Forest Ecology and Management.

to habitat loss and climate change in the future (Higgins and Richardson, 1999).

Today, increased human population size imposes an escalating challenge to provide food and fiber. Concomitant with human population increase is deforestation and decrease in forested lands. *Pinus taeda* is the dominant coniferous species in the southern quadrant of the United States providing a major source of wood and fiber (Shultz, 1999). The *P. taeda* geographic range and population size may have shifted and changed during the past 21,000 years (the Late Quaternary; Late Pleistocene through Holocene) in response to climate change and anthropogenic factors. These changes are believed to have caused a significant imprint on the species genetic structure.

To test these assertions, the past 21,000-year period was divided into three time scales. These time scales were determined based on the available tools, information and resources by which changes to *P. taeda* populations during these time scales can be tested. These time scales are the geological, the historical and the recent time scales. Prior to discussion of these time scales, a description of the phylogenetics, taxonomy and early evolution as well as a description of the life history attributes of the species is appropriate.

Phylogeny, taxonomy and early evolution of *Pinus taeda*

Taxonomy

Pinus taeda L. belongs to the genus *Pinus*, the largest genus among the conifers and the most widespread genus of trees in the northern hemisphere (Critchfield and Little, 1966). The genus *Pinus* is divided into two main groups: subgenus *Strobus* (*haploxylon* or soft pines) and subgenus *Pinus* (*diploxylon* or hard pines) to which *P. taeda* belongs (Critchfield and Little, 1966; Mirov, 1967; Miller, 1977; Price et al., 1998; Wolf et al., 2000). There are 10 hard pine subsections and *P. taeda* belong to the subsection *Australes* (Price et al., 1998).

Today, *P. taeda* range extends across a large geographical area. The easternmost range of the species is southern Delaware (39^{o} 21' N) through Maryland, Virginia, North Carolina, South Carolina, Georgia into central Florida then westward through Alabama, southern Tennessee and eastern Mississippi. The Mississippi River Valley (MRV) marks the western range of *P. taeda*. Louisiana, southwestern Arkansas, southeastern tip of Oklahoma, eastern Texas and the remote disjunct pine islands known as the Lost Pines constitute the western range of the species (Baker and Langdon, 1990).

Early evolution

The genus *Pinus* is believed to have originated in the early-middle Mesozoic (Table 1.1) at mid-latitudes (Miller, 1977; Millar, 1993; Millar, 1998) and then spread through migration events to the east and to the west (Millar, 1993; Millar, 1998). Late Cretaceous was characterized by climatic quiescence although it was warmer by 10 to 20 °C than present-day temperatures and more humid as well. *Pinus* is believed to have expanded during this period. The early Tertiary, 65 million years ago (MYA), was characterized by major climatic changes that profoundly affected the evolution of pines. Temperatures were warmer by five to seven °C and more humid than Cretaceous, reaching a maximum in the Eocene (54 MYA). Due to these warmer temperatures, Eocene was characterized by the formation of pine refugia (Millar, 1998). Pines may have expanded and contracted following the cooling-warming cycle. This cycle is opposite to recent Pleistocene cycles of expansion and contraction in the sense that, during Eocene, pines may have been restricted to refugia during warmer periods and expanded during cooler periods (Millar, 1998).

Table 1.1

Approximate ages and durations of geological eras from the Mesozoic to present-day*

Era	Period	Epoch	Duration (millions of years)	Millions of years ago
Cenozoic	Quaternary	Holocene		0.01
		Pleistocene	2.4	2.5
	Tertiary	Pliocene	4.5	7
		Miocene	19	26
		Oligocene	12	34
		Eocene	16	54
		Paleocene	11	65
Mesozoic	Cretaceous		71	136
	Jurassic		54	190
	Triassic		35	225

* Adopted from Millar (1998).

After the Eocene came the Oligocene. In the Oligocene (34 MYA), temperatures are believed to have dropped 10 to14 °C, rainfall may have decreased and seasonality may have increased from two to five °C during the Eocene to 25 °C during the Oligocene (twice the current seasonality). Complex continental climate as well as ice sheets may have possibly formed during this period for the first time. Extensive volcanic activity and mountain building also occurred. Pines may have colonized new areas during those conditions through the Miocene (26 MYA) (Millar, 1993; Millar, 1998). From this short summary of the evolution of *Pinus* in the New World, it appears that the interaction of expansion, contraction, isolation, refugia, migration and gene flow factors shaped the evolution of pines.

Australes is believed to have existed in western North America and northern Mesoamerica during the Eocene and then radiated from there subsequently eastward (Axelrod, 1986; Krupkin et al., 1996; Millar, 1998; Lopez et al., 2002). It has been suggested that the *Australes* and *Oocarpae* subsections have diverged 10 to 12 MYA from an *Oocarpae*-like pine that occupied northern Mexico/southwestern US (Krupkin et al., 1996; Dvorak et al., 2000). It appears that *Australes* and *Oocarpae* diverged when they migrated along the northern Gulf coast of the United States. During a five to ten million year period of evolution, the first pine to separate from this complex was hypothesized to be *Pinus palustris* (long-leaf pine) (Dvorak et al., 2000). The most recent species to diverge from the subsection *Australes* (continental) is believed to be *P. taeda* (Krupkin et al., 1996; Dvorak et al., 2000).

Pinus taeda populations occur in two large regions, east and west of the Mississippi River Valley (Fig. 1.1). The westernmost and disjunct Texas population of *P. taeda* is known as the Lost Pines (Fig. 1.1). The genetic structure of eastern and western populations of *P. taeda* along with the genetic structure of the Lost Pines will be investigated in this dissertation.

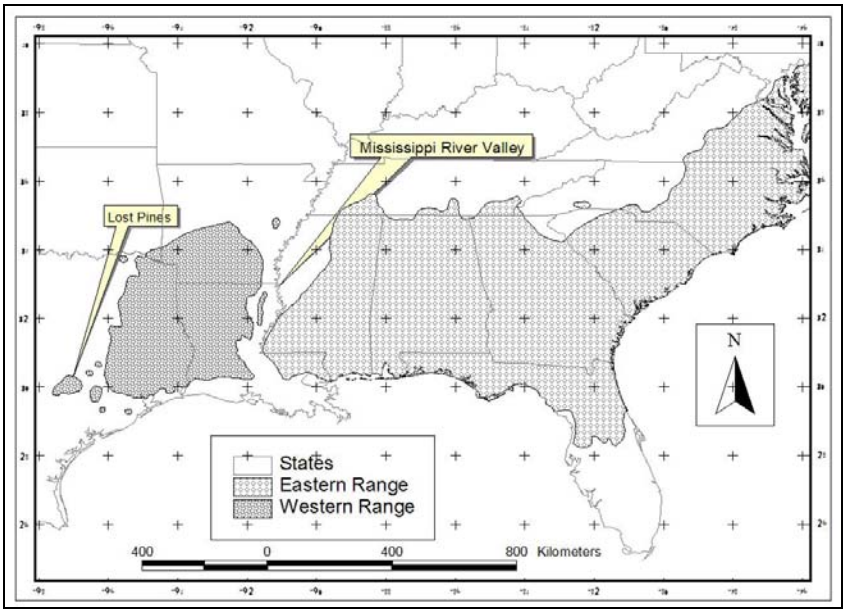

Fig. 1.1. Geographic range and distribution of *Pinus taeda* L. showing the eastern and western parts of the range with respect to the Mississippi River Valley along with the disjunct westernmost populations of the Lost Pines (modified after Critchfield and Little, 1966).

Although the Tertiary events (65-2.5 MYA) are believed to have impacted the evolution of pines profoundly, Quaternary (Pleistocene and Holocene) events (2.5 MYA-present) are also of importance to the study of genetic structure of pine populations (Millar, 1998). Given the recent evolution of *P. taeda* and the available resources, this study will focus on the response to events of the late Quaternary (21,000 years before present or YBP). The late Quaternary climatic events thought to have affected the dynamics of *P. taeda* will be divided into three time scales and discussion follows in a later section.

Life history of *Pinus taeda*

The knowledge of the life history attributes of *P. taeda* is important. It helps in interpreting and discussing the results of this study in an appropriate context. These attributes are of particular importance for interpreting present-day population genetic structures of *P. taeda* as well as inferring the past events shaping this structure.

Pinus taeda is a long-lived woody perennial plant which aggressively colonizes in open areas early in succession through heavy annual seed production. Seed dispersal falls mainly within the nearest 60 to 90 meters (m) from the source tree (Baker and Langdon, 1990) but rare long distance seed dispersal is possible (Richardson et al., 1994) to the extent that local populations are interconnected as a panmictic network. *Pinus taeda* L. (Pinaceae) is wind pollinated and monoecious. Pollen dispersal is possible as far as few hundreds of kilometers (km) (Bessy, 1883) especially under suitable temperatures and wind conditions. The catkins (pollen-bearing male structures) are usually lower in the tree strata than the female strobili (ovule-bearing female structures). The catkins usually ripen before the female strobili, enabling cross-pollination and limiting self-pollination (Wahlenberg, 1960; Schultz, 1997). It appears also that

the embryo lethal system may also interfere to promote cross-fertilization (Bramlett and Popham, 1971; Koski, 1971).

A life history attribute that plays an important role in the interpretation of population structure is the generation interval. This is the time interval between germination and reproductive onset. *Pinus taeda* rarely produces female strobili as early as four to five years of age (Dorman, 1976; Schultz, 1997) but trees become fully productive at the age of 10 to 15 years in open stands and possibly later in closed stands (Shultz, 1997).

Regeneration of wild stands is predetermined by the presence of suitable open areas. This is important to a shade-intolerant colonizer like *P. taeda*. Open areas can be obtained mainly by fire (Quarterman and Keever, 1962) or other types of factors that creates gaps in the forest, like lightening strikes, falling dead trees, or wind. Recently, forest clearing for agricultural activities became another major cause for creating openings in the forest and enhancing pine regeneration when these areas are abandoned (Streng and Harcombe, 1982; Schafale and Harcombe, 1983; Williams, 1989 pp. 466-477).

In fire-prone areas, generation interval may be longer than 10 to 15 years but shorter than 30 years based on seedling survival (Pyne et al., 1996). Pre-settlement fire history in east Texas shows that major fires occurred in a cycle

of less than 35 years (Wade et al., 2000) which support the longer generation interval of 15 to 30 years for *P. taeda* forests in Texas.

Chronology of time scales and evolutionary events

The geological time scale

In this study, the geological time scale spans the time since the peak of the Last Glacial Maximum (LGM) (21,000-14,000 YBP) through the onset of the Holocene (11,000 YBP) to the mid-Holocene (8,000-5000 YBP) and ending by late-Holocene (5,000 YBP-present).

The Southern pine species (which includes *P. taeda*) is believed to have been confined to uncertain refugia at the peak of the LGM (21,000-14,000 YBP) (Jackson et al., 2000). The confinement of the southern pines is believed to be a response to an advancing glaciers reaching into the northern United States. Glaciers advancement is believed to have caused a major climatic shift and consequently caused a major shift in the distribution of flora and fauna (Davis and Shaw, 2001).

Fossil pollen for southern pine species has been found in southern Florida indicating the presence of a refugium; however, the northern extent of the

refugium was not determined (Jackson et al., 2000). Fossil pollen for southern pines has also been found in a central Texas bog dating back to 16,000 YBP (Bryant and Holloway, 1985). The affinity of fossil pollen records at the species level cannot be determined definitively because there were other close relatives in the same area. The confinement of *P. taeda* to a refugium or two refugia cannot be ruled out by fossil pollen so it is possible that *P. taeda* was confined to one or two refugia in southern Florida and/or southern Texas based on paleo-climatic analysis.

In support of a southern Texas refugium, other studies have showed that floristic exchange between southeastern United States and northern Mexico was possible during the Pleistocene and Tertiary. The last vegetation continuum and floral exchange is hypothesized near the LGM (Dressler, 1954) although Tertiary exchange was favored to explain distribution and biogeography of floristic species (Steyermark, 1950; Martin and Harrell, 1957). Even for those who favored the Tertiary floristic exchange, they could not exclude the possibility of Pleistocene exchange (Perry et al., 1998).

In Texas, the LGM was characterized by cooler temperature as much as four to six °C or more with increased moisture. In east and central Texas, vegetation has been inferred to be open mixed conifer-hardwood woodland with some boreal elements, an expected outcome of the cooler temperatures prevailed at

the peak of the LGM (Graham and Heimsch, 1960; Barry, 1983; Bryant and Holloway, 1985; Toomey et al., 1993; Waters and Nordt, 1995; Musgrove et al., 2001). The winter temperatures may have not been considerably cooler than present-day, but summer temperatures were at least five °C cooler (Holloway and Bryant, 1984; Bryant and Holloway, 1985; Stute et al., 1992; Toomey et al., 1993).

Gulf Coast sea level was 70 to 140 m lower than the present-day which caused part of the continental shelf to be dry land (Bloom, 1983). By late-Glacial (14,000-10,000 YBP), temperatures were two to three °C cooler with moisture first decreased and then increased (Toomey et al., 1993).

Glaciers at the northern United States retreated to northern latitudes starting 14,000 YBP (the late-Glacial) due to a warming trend. The Laurentide ice sheet retreated largely from the United States by 11,000 YBP marking the onset of the Holocene as a warm period (Mickelson et al., 1983; Porter, 1983). When glaciers retreated, warm-temperate pine forests (which include *P. taeda*) are believed to have colonized as far northward as climate conditions permitted. This pattern of glaciers advancement and retreat is thought to have happened as many as 20 times in the Pleistocene over the past two million years (Butzer, 1976) implying a potentially significant dynamics of expansions and contractions in the population size and geographic range of *P. taeda*. The

alternative scenario is that the range of *P. taeda* was altered little if at all because climatic changes were not severe enough for large-scale shifts in species range.

In central and south-central Texas, where the Lost Pines are located today, the warming-drying trend may have culminated in the mid- to late-Holocene (8,000-2,500 YBP) and more precisely between 5,000-2,500 YBP (Toomey et al., 1993; Waters and Nordt, 1995; Brown, 1998; Goodfriend and Ellis, 2000). The period of the Holocene when the weather was much warmer and drier than now is known as the Altithermal or the Xerothermic period (Bryant, 1977; Toomey et al., 1993; Waters and Nordt, 1995; Bousman, 1998; Wilkins and Currey, 1999).

The Xerothermic period might have caused the isolation and disjunction of flora in Texas and Mexico (Dressler, 1954) as well as other areas like California (Axelrod, 1981). These disjunct flora are important for inferring past plant migration events (Braun, 1955; Axelrod, 1981), hence the importance of the Lost Pines of central Texas.

The historical time scale

The historical time scale in this study refers to the time scale of written historical records and accounts of early explorers and settlers (approx. 500 YBP in central Texas). This time scale is focused on the Lost Pines of central Texas and the western range of the species (Fig. 1.1). Human habitation in Texas spans several thousands of years (Maynard, 1955; Newcomb, 1961; Hester and Turner, 2003) but there are no written records until the arrival of early Spanish explorers.

Central Texas Native Indian American (Tonkawa) records and artifacts showed little to non-existent agriculture and forest clearing activities (Maynard, 1955; Newcomb, 1961 pp. 135; Hester and Turner, 2003). They relied on hunting, gathering and fishing but did not practice agriculture (Newcomb, 1961 pp. 135). This restricts the most significant of the human impact on the Lost Pines to the time scale of early European and Anglo-American settlers. Records of settlers frequently mentioned the Lost Pines.

Spanish exploration of Texas started as early as 1519 when Alonso Alvarez de Pineda explored the Texas Gulf Coast only with no inland exploration (Chipman, 2003). This was followed by several attempts like the 1541 exploration of west Texas by Francisco Vazquez de Coronado (Chipman,

2003). After the occupation of New Mexico in 1598, exploration of Texas became more extensive. Between 1629 and 1654, expeditions from New Mexico entered Texas to search for Indians and to establish trade with them (Chipman, 2003). In 1690, an expedition by De Leon and Mansanet went through Fayette County where part of the Lost Pines is located today. One year later, the next expedition in 1691 by Teran de los Rios went along the Colorado River where other parts of the Lost Pines are also located today.

Anglo-Americans were first invited into Texas in the late 1770's by the Spanish government in order to populate Texas after it failed to convince its own people to do so (Henson, 2003). Populating Texas, according to the Spanish government, would increase economic development and deter Plains Indians like the Comanches and Kiowas (Moore, 1977; Henson, 2003). In 1820 just one year before Mexico gained independence, Anglo-American settlements started. The Mexican government continued to allow the Anglo-American settlements in Texas in exchange for loyalty under oath (Henson, 2003).

At the same time, Anglo-Americans were attracted into Texas for two reasons according to Henson (2003). First, the settlers thought that east Texas would be purchased by the United States (some thought that Texas was part of the Louisiana Purchase) and immigration would be enhanced and thus their land values would increase. Second, there was no reciprocal agreement between

the United States and Mexico to collect debt or to return fugitives. Therefore, Texas was a safe place for farmers who were not able to pay their loans and debts after the end of the war of 1812. Texas was appealing because settlers could reestablish themselves and start over after a financial loss (Henson, 2003).

An indirect measure of the impact of human disturbance through logging can be measured by the population growth in Texas. The population of Texas grew from 1,500 in 1744 to approximately 3000 by 1792 to around 7,000 by 1806. This number was not much greater 15 years later when Stephen F. Austin founded his colony on the Brazos River (Bastrop Historical Society, 1955). In 1826 the Austin colony, near the Lost Pines, contained a population of 1,800 which grew to 24,700 during the colonization period of 1821 to 1834. In 1836, there were probably close to 50,000 Anglo-Americans, Blacks, Hispanics, and Indians in Texas (CENSUS ’90, 1990).

Between 1870 and 1880, the settler population in the entire state of Texas approximately increased 95 percent to reach a total of 1.5 million (CENSUS ’90, 1990). For Bastrop County, population census reached 11,000 by 1870 and peaked to 26,845 by 1900 (Henry, 1902).

Expanding human settlements increased the demand for timber (Bastrop Historical Society, 1955; Marks, 2003). Historical records showed the importance of *P. taeda* to the early settlers at the western edge of the species (Easton, 1947; Bastrop Historical Society, 1955). Logging may have or may have not been a recent factor in changing the range and size (dynamics) of the Lost Pines. Despite heavy logging, estimates of the Lost Pines population size were almost the same between the mid 19th century and the mid 20th century (Easton, 1947; Anonymous, 1952). Easton (1947) quoting an estimate of the Lost Pines area mentioned by Sargent (in 1880 Census) that the Lost Pines near Bastrop "had covered four townships, or about 90,000 acres". The Texas Forest Service estimated in 1952 that the Lost Pines still covered an area of about 85,000 acres (Anonymous, 1952). Up until the mid 20th century, logging companies produced sustainable amount of lumber from the Lost Pines area annually (Easton, 1947).

The historical time scale of the study also focuses on the origin of the Lost Pines in light of historical records of early settlers and explorers. The Lost Pines population is well-documented as a natural occurrence, not as a planted forest. Spanish explorers, Anglo-American settlers, naturalists and geologists reported pines in central Texas (Buckley, 1866; Kennedy, 1925; Anonymous, 1952; Fitzwilliam, 1955; Youngman, 1965).

The recent time scale

The recent time scale, from 1950 to 1995, is focused on the distribution and dynamics of the Lost Pines during this period. Between 1933 and 1937, Bastrop State Park and Buescher State Park were acquired from private landowners as state parks. These two state parks contain large portions of the Lost Pines population. Logging ceased in these two parks after the 1930's which provided a unique opportunity to infer the impact of logging prior to the 1930's by studying the absence of logging on the Lost Pines after park acquisition. This unique opportunity was made possible by aerial photographs for the two state parks from 1949 to 1995. The availability of soil maps also provided an opportunity to study relationship between soil characteristics and the distribution and dynamics of the Lost Pines.

How to reconstruct the natural history of *Pinus taeda*?

Attempts to reconstruct population dynamics and natural history of pines and other species in response to climatic change are abundant in the literature (Raja et al., 1997; Allnut et al., 1999; Ledig, 1999; Ledig, 2000; Jackson and Booth, 2002; Premoli et al., 2002; Burban and Petit, 2003; Hoffmann et al., 2003; Llewellyn et al., 2003). Different approaches have been used to reconstruct past events such as fossil records (pollen data or macro fossils),

geological history, morphological or biochemical phenotypes, biochemical markers and DNA markers.

The nuclear genome is a rich source of genetically informative variation (Schaal et al., 1998). It is a bi-parentally inherited genome allowing for recombination and heterozygosity. Recombination was once viewed as a drawback for phylogenetic studies (Avise, 1998; Newton, 1999) but nuclear genome still is the predominant and most informative genetic material used in population genetics (Zhang and Hewitt, 2003).

The use of DNA markers especially microsatellites has proven powerful for reconstructing natural history (Jarne and Lagoda, 1996; Goldstein and Pollock, 1997). Nuclear microsatellites are a tandem simple sequence repeats (SSR) of DNA that are highly variable (Li et al., 2002). In general, these tandem repeats can change in number and structure due to mutation which will cause the number of repeats to be either higher or lower in number (Kimmel and Chakraborty, 1996; Schlotterer, 2000). The polymerase chain reaction (PCR) can amplify these DNA segments with a specific primer set causing the DNA length variability to be amplified too. These different PCR products can be separated on a gel as different bands indicating both indels in the flanking regions and differences in repeat unit number (Schlotterer, 2000).

Microsatellite markers are co-dominant which means they can resolve the heterozygous state, providing an excellent tool for population genetic studies and DNA fingerprinting (Smouse and Chevillon, 1998). Homoplasy; however, can produce the same bands on the gel while these bands may not be identical in sequence (Estoup et al., 2002). This is the major drawback of using microsatellite markers (Estoup et al., 2002).

Population dynamics at large or small spatial or temporal scales can leave signatures that can be detected with the appropriate molecular tools. DNA signatures are revealed by the analysis of genetic variation. Analysis of genetic variation among contemporary individuals and populations can provide insights into prehistoric events (Barbujani and Bertorelle, 2001; Waldick et al., 2002; Cavalli-Sforza and Feldman, 2003). DNA signatures in this study refer to the collective information stored as alleles and genotypic frequencies at the individual and population levels which can be detected and interpreted in statistical models.

Allele and genotype frequencies in a population are the product of selective forces. For example, if a population was founded by a single individual or few individuals, then the DNA signature will be a founder effect. The presence of a founder effect can be detected and tested using appropriate genetic tools and analyses (Ledig, 2000). Or, if populations were separated from each other,

then isolation by distance might be another type of DNA signature detected in these populations. Of a particular interest to the Lost Pines is using DNA signatures to detect a bottleneck. A bottleneck is defined as a sudden reduction of the population size (Nei et al., 1975; Maruyama and Fuerst, 1985). Bottleneck signatures as important DNA signatures will be discussed in detail in later chapters.

Since DNA signatures are functions of alleles and genotype frequencies, they are transmitted from one generation to another. Consequently, generation interval plays an important role in aligning temporal events with population dynamics. For long-lived tree species such as *P. taeda* with a generation interval of 15 to 30 years, the resolution of the DNA signatures can reveal past plant population changes as far back as thousands and even millions of years.

Aerial photographs for the Lost Pines between 1949 and 1995 furnish other methods for studying population changes over a recent time scale. Recent technologies such as image analysis, remote sensing and geographic information systems (GIS), along with the availability of aerial photographs, weather and soil data provide invaluable resources to examine the dynamics and distribution of the species at the recent time scale. Combining DNA analysis and spatial sciences creates a unique time-scale continuum for studying the Lost Pines.

The case is made that the genetic consequences for population dynamics after Pleistocene glaciation can be detected in current patterns of allelic variation. *Pinus taeda* is an ideal candidate for such studies because its range may have expanded and contracted due to climate change over the past 21,000 years. Given the geological history of the south and southeastern United States since the onset of the last glaciation event and the recent history of the western edge of the species including the Lost Pines, a few questions remain to be answered to reveal geological, historical and recent dynamics in response to these forces. These questions are related to the distribution, dynamics and genetic structure of this species which can be addressed using DNA signatures via nuclear microsatellites, historical records and image analysis. Image analysis includes remote sensing, GIS and spatial statistics. These questions are as follows:

1) How does the Lost Pines population fit within the present-day natural range in terms of its allelic richness?

2) Is there evidence that the western range for *P. taeda* has changed since the time of glaciation, especially with respect to climate change over a geological time scale?

3) What can we conclude about the origin and dynamics of the Lost Pines from microsatellite markers?

4) What are the impacts of logging and/or the effect of soil characteristics on the Lost Pines in the 20th century?

These four questions will be sequentially addressed as testable hypotheses in the following subheadings.

1) How does the Lost Pines population fit within the present-day natural range in terms of its allelic richness?

The first question is related to the current distribution of the species and its genetic structure due to ancient dynamics. This question should be asked in the context of the geological and life history attributes of the species.

The distribution of the species east and west of the Mississippi River Valley is an interesting phenomenon. These two large populations are physically isolated from each other by the effect of the floodplain of the Mississippi River by a distance of 50 to 200 km. This physical isolation is accompanied by a significant but modest differentiation at the morphological, biochemical and DNA levels (Wells and Wakeley, 1966; Florence and Rink, 1979; Wells et al.,

1991; Schmidtling et al., 1999; Williams et al., 2000). This separation and differentiation can be attributed to two different models.

The first model is called the two-refugia model. It suggests that *P. taeda* was restricted to two refugia east and west of the Mississippi River during the Last Glacial Maximum (LGM) 21,000 years ago using morphological data (Wells and Wakeley, 1966; Wells et al., 1991) and isozyme data (Schmidtling et al., 1999). These two refugia were kept separated long enough to evolve separately in the absence of gene flow. These two populations expanded when the glaciers retreated at the beginning of the Holocene (Schmidtling et al., 1999) which allowed for recent gene flow.

The other model is called the restricted gene flow model. Florence and Rink (1979) suggest the presence of only one refugia east of the Mississippi River, and that the population expanded westward when the glaciers retreated. The model suggests that the populations east and west of the Mississippi River were then separated by the floodplains of the Mississippi River and that these floodplains caused gene flow to be limited which might have caused the differentiation. This model was supported by their isozyme data which showed that diversity and genetic structure was partitioned between east and west of the Mississippi River Valley. The isozyme data from Florence and Rink (1979) is provided in Appendix (A) because it is difficult to locate.

The previous two models can be tested using nuclear microsatellites. Gene flow is a significant factor that shapes the allelic structure of populations. Gene flow via pollen dispersal is extensive because pine pollen can move over 600 km in the planetary boundary layer 10 km above ground (Bessey, 1883; DiGiovanni et al., 1996).

The two previous studies provided useful insights into the genetic structure of *P. taeda* but also provided a contrasting explanation. Florence and Rink (1979) reported an overall mean number of alleles (MNA) of 3.6 alleles/locus for six isozyme markers using 205 samples collected from 8 major regions across *P. taeda* range; however, part of these samples are bulk collections. Thirty nine samples were collected from central Texas including the Lost Pines. From the data in Appendix (A), the western populations possess genetic diversity similar to the eastern populations. Florence and Rink (1979) use correlation and regression techniques to test relationship between geographic location and climatic conditions to genetic diversity. They also use private allele distribution to indicate differentiation.

Similarly, Schmidtling et al. (1999) reported MNA of 3.8 alleles/locus using 18 isozyme markers and different sources of plant material with larger sample size but no samples from the Lost Pines were collected. Genetic diversity for

populations west of the Mississippi River was lower than for populations east of the Mississippi River (Schmidtling et al., 1999). Regression analysis and distribution of private alleles were used to assess genetic structure of populations across geographic locations (Schmidtling et al., 1999).

Both isozyme studies reported higher diagnostic alleles east of the Mississippi River compared to west of the Mississippi River. However, the two studies each concluded with a different phylogeographic model for *P. taeda*.

A third study based on 18 nuclear microsatellites used a sample size of five from the Lost Pines because the study was designed for other purposes (Williams et al., 2000); however, the study results agreed in general terms with the results of the previous studies. Chord genetic distance, principal component analysis and private allele distribution were used to infer population structure and differentiation. Lower allelic diversity was reported for the Lost Pines; however, from the principal component analysis, Williams et al. (2000) concluded that the Lost Pines and samples from east Texas share a common ancestor.

In this recent microsatellite study, we increased the sample size to compare *P. taeda* allelic diversity and diagnostic alleles between the eastern and western

parts of the species range. Chapter II will address this question. Partial answers of these questions can also be found in Chapter III and IV.

2) Is there evidence that the western range for P. taeda has changed since the time of glaciation, especially with respect to climate change over a geological time scale?

The second question is related to the dynamics of the Lost Pines at the western edge of the species natural range. Post-Pleistocene studies have focused on population expansion as boreal species returned to higher latitudes after glaciation (Davis and Shaw, 2001). The genetic consequences for the contraction of a species range have been far less studied (Davis and Shaw, 2001). The genetic consequences of the population contraction provide some insight about the fate of marginal populations in the face of global warming and climate change.

The distribution, dynamics and genetic structure of the Lost Pines in central Texas are well-suited to the study of Holocene population contraction of *P. taeda* range. Lost Pines in central Texas occur in a region of North America, which may have been a glacial refugia or suture zone (zone of secondary contact between previously isolated biotas) (Remington, 1968). Combined with its rich historical framework, this region can provide explicit hypotheses about

the genetic consequences of population contraction for a warm-temperate conifer, *P. taeda*. Hypotheses about this isolated population can be tested. We have developed two testable hypotheses about this isolation: the population contraction hypothesis and the steady-state hypothesis.

Population contraction hypothesis

A bottleneck is defined as the sudden reduction of population size (Nei, 1975). The effective population size (N_e) is the size of an idealized population that meets the conditions of Hardy-Weinberg Equilibrium: no mutation, no migration, no selection, infinite population size and random mating. The effective population size is adjusted for one or more deviations from idealized conditions. In most situations, the effective population size (N_e) is lower than census population size (N). If the reduction in population size changed the census population size (demography), then a demographic bottleneck has occurred (Spencer et al., 2000). If this reduction in the census population size changed and reduced the effective population size (N_e) then a genetic bottleneck has occurred (Luikart, 1997).

The impact of the genetic bottleneck depends on the size of the population before, during and after the bottleneck event. A demographic bottleneck may or may not cause a genetic bottleneck depending on the effective population size

(N_e) at the time of the bottleneck. A demographic bottleneck certainly happens when a population is reduced from $N_e=10^6$ individuals to $N_e=10^5$ individuals, but $N_e=10^5$ individuals may be large enough to prevent a genetic bottleneck. When N_e decreases to low values (less than 1000 for example), there is a large stochastic effect on allele frequency. This stochastic effect is more dramatic for smaller N_e.

We hypothesize that a series of pine island extinctions and recolonizations occurred in central Texas on a geological time scale during the Holocene. This hypothesis is suggested by paleo-climate records. Palynological analysis showed that the climate transition in central Texas moved gradually from cool, cool-moist, warm-dry, and warm-moist to warm-dry climate over the past 12,500 years (Graham and Heimsch, 1960). The transition from cool to cool-moist could have caused a series of population expansions followed by a contraction that started 8000 years B.P. and culminated during the peak of the Altithermal (Xerothermic) period between 5,000 and 2,500 years B.P. (Toomey et al., 1993). A shift towards the warm-moist climate may have provided for expansion of the pine forest (2,500-1,000 years B.P.) followed by a gradual contraction in response to a warm-dry climate in more recent times from 1,000 years B.P. to the present time (Bryant, 1977; Bryant and Holloway, 1985; Toomey et al., 1993; Waters and Nordt, 1995; Bousman, 1998; Brown, 1998; Russ et al., 2000; Musgrove et al., 2001). Evidence supporting this climate shift

has been extracted from pollen analysis, hydrology of rivers, speleothems, isotopic studies and other types of archeological or fossil evidence.

One of the studies is based on oxalate residues from lichens (Russ et al., 2000). It shows that mid- to late-Holocene period was itself a series of xeric and mesic periods revealing five episodes of xeric conditions from 6,400 to 700 years B.P. (Russ et al., 2000). During the late Holocene, xeric conditions have prevailed for the past 1,000 years (Russ et al., 2000).

Steady-state hypothesis

Specific edaphic conditions in central Texas are hypothesized to have constrained the past and present range of the Lost Pines. Under this hypothesis, there was no real expansion or contraction of pines in central Texas during the Pleistocene or the Holocene (Patty, 1968). This hypothesis is based on the assertion that, in central Texas, *P. taeda* can only grow on specific soil types, mostly sandy to sandy-loam surface soils with heavy clay subsoils. Given the limited availability of such soils and the time of their formation, the Lost Pines population may have been roughly the same size for about two million years ago or more (McBryde, 1933; Silker, 1973).

According to the steady-state hypothesis, Lost Pines should have less allelic richness (Babbell and Selander, 1974) when compared to similar *P. taeda* populations further to the east. No sign of a genetic bottleneck is expected since effective population size of the Lost Pines would not have contracted.

Genetic consequences of these two proposed hypotheses can be tested using microsatellite markers and mutation-drift equilibrium (Cornuet and Luikart, 1996). Chapter III addresses this question.

3) What can we conclude about the origin and dynamics of the Lost Pines from microsatellite?

It is important to examine the evidence supporting the natural origin of the Lost Pines population. Earlier writers suggested that *P. taeda* seeds could have been brought by early explorers, Indians, birds as well as natural means into central Texas (Anonymous, 1952; Bastrop Historical Society, 1955; Wilson, 1962; Youngman, 1965; Moore, 1977). DNA markers can authenticate or refute these reports. This will serve as a foundation for testing hypotheses on a geological time scale in central Texas. Introduction or admixture can be tested using microsatellite markers.

Morphological evidence suggests that the Lost Pines resemble east Texas pines but that physiologically the Lost Pines are more drought resistant (Zobel and Goddard, 1955; Ursic, 1961; Thames, 1963; Youngman, 1965; van Buijtenen, 1966; Knauf and Bilan, 1974). This finding has prompted studies of needle and foliage traits. Needle length of the Lost Pines is not significantly different from needle length in other parts of Texas; however, needle cross-sectional area and perimeter are significantly higher than Texas material (Thames, 1963). The Lost Pines also show lowest number of stomata per unit length among other Texas pines (Thames, 1963).

Lost Pines were in central Texas 300 to 400 years ago although Sargent (see maps in Williams, 1989) showed no Lost Pines population in central Texas by the late 19th century. Microsatellite markers can be used to ascertain the origin of this population (Smouse and Chevillon, 1998; Banks and Eichert, 2000). Multivariate analysis utilizing the multi-locus genetic information can also be used to discern between the possible origins of a population (Cavalli-Sforza et al., 1994). Chapter IV will address this question.

4) What are the impacts of logging and/or the effect of soil characteristics on the Lost Pines in the 20th century?

The fourth question addresses Lost Pines dynamics during the recent time scale. Recent population dynamics can be related to logging followed by an absence of logging. Another part of the question is related to the steady-state hypothesis where specific soil characteristics may have constrained the distribution of the Lost Pines. Aerial photographs, soil maps and climate records on the recent time scale (1949-1995) provide a unique source of information on the Lost Pines populations in relation to soil characteristics. Bastrop State Park and Buescher State Park in Bastrop County, Texas, encompass most of the present-day Lost Pines population in central Texas. Aerial photographs dating back to 1949 can be compared with recent aerial photographs (1995) to assess the Lost Pines population dynamics during the past 45 years. Pine distribution can be determined from remote sensing imagery (aerial photographs and satellite images) then overlaid with soil maps, a GIS format to test the steady-state hypothesis that pines in Bastrop County are restricted to a soil type.

Historical records from the early settlers in central Texas showed that the Lost Pines were a major source of timber for the development of this area (Bastrop

Historical Society, 1955). The Lost Pines area was extensively logged around 1880 (Easton 1947; Bastrop Historical Society, 1955).

The steady-state hypothesis may explain the geographic isolation of the Lost Pines based on the limited availability of appropriate soil characteristics (McBryde 1933; Silker, 1973). Sorenson et al. (1976) hypothesized that the Lost Pines expanded westward during ameliorative conditions but were confined to terraces along the Colorado River from east Texas up to the Edwards Plateau. The presence of clay soils surrounding those pines prevented their expansion further to the west or to the south but climatic condition may have permitted forest expansion (Sorenson et al., 1976). Chapter V addresses these questions.

The relationship between the Lost Pines and the soil characteristics can be inferred from the soil characteristics of Bastrop State Park. The park soils belong mainly of the Axtell-Tabor association which is characterized by the presence of a gravelly sandy loam or fine sandy loam surface layer approximately 35 to 45 cm thick with a red mottled clay subsoil (Baker, 1979). Ground water levels in Bastrop County vary from one m to about 66 m below soil surface in various places in Bastrop County during different times of the year (Swartz, 1956; Follett, 1970). Water level in and around the Lost Pines area varies from shallow to deep (Swartz, 1956; Follett, 1970).

Bastrop County is located on the 97th meridian west. The climate is humid and sub-tropical (Moore, 1977; Baker, 1979). Air-masses dominate the weather of Bastrop County providing tropical maritime in the spring, summer and fall and polar air masses in the winter. These air masses provide a continental climate characterized by considerable variation in temperatures (Baker, 1979). Average annual rainfall is approximately 940 mm (Baker, 1979). Rainfall occurs mainly in the form of thunder showers and peaks in June and September (Baker, 1979).

CHAPTER II

POPULATION DYNAMICS OF *Pinus taeda* L. BASED ON NUCLEAR MICROSATELLITES

Overview

Pinus taeda has historically large, interconnecting populations which extend along the U.S. Atlantic seaboard from Maryland to Florida and westward to central Texas. Genetic divergence is greatest east and west of the Mississippi River Valley but there are no estimates of allelic diversity or diagnostic alleles. To test allelic diversity and detect diagnostic alleles, samples were drawn from grafted archives of natural stand selections from 1950 to 1970. The samples represented *P. taeda* prior to intensive plantation establishment and domestication.

The eastern range had higher mean allelic diversity than western populations (10 versus 8.5 alleles per locus) even after adjustment for sample size and geographic area. A total of 46 unique alleles were detected; nine were considered diagnostic. There were four times more diagnostic alleles east of the Mississippi River Valley compared to the western part of the species' range. Gene flow estimates for *P. taeda* were high, ranging from two to six migrants per generation per population. Cross-validation using Mississippi and

Alabama samples supported the gene flow patterns, showing an admixture at the juncture of the Mississippi River Valley. Lower allelic diversity in the western range of *P. taeda* and higher number of diagnostic alleles in the eastern range are attributed to 1) a prevailing wind direction which facilitates viable pollen movement from west to east and 2) a gradual contraction in western *P. taeda* population sizes over the past 5,000 to 8,000 years.

Introduction

Interest in population dynamics at a landscape level has renewed interest in forces shaping population structure. The genetic structure of conifers has been shaped by climate changes, aggressive colonization, slow onset of reproduction and an outcrossing mating system. *Pinus taeda* presents an interesting case study because it has a continuous distribution throughout the southern United States. Sampling *P. taeda* east and west of the Mississippi River Valley shows pronounced genetic differentiation at morphological and isozyme levels (Florence and Rink, 1979; Wells et al., 1991; Schmidtling et al., 1999). With hypervariable microsatellite markers, allelic diversity and diagnostic alleles can be estimated between *P. taeda* populations east and west of the Mississippi River Valley.

Pinus taeda L. (Pinaceae) is wind-pollinated and monoecious. The easternmost range of the species is southern Delaware (39^{o} 21' N) through Maryland, Virginia, North Carolina, South Carolina, Georgia into central Florida then westward through Alabama, southern Tennessee and eastern Mississippi. The Mississippi River Valley delineates the western range of *P. taeda*. Louisiana, south-western Arkansas, south-eastern tip of Oklahoma, eastern Texas and the remote pine islands known as the Lost Pines constitute the western range of the species (Baker and Langdon, 1990). Seed movement is more limited than pollen dispersal. *Pinus taeda* disseminates seeds only 61 to 91 meters downwind and 23 to 30 meters in the other directions (Baker and Langdon, 1990). Gene flow via pollen dispersal is far more extensive because pine pollen can move over 600 km in the planetary boundary layer 10 km above ground (DiGiovanni et al., 1996).

Two previous studies of *P. taeda* population structure have been conducted using isozyme markers but no estimates of allelic diversity or diagnostic alleles were reported (Florence and Rink, 1979; Schmidtling et al., 1999). A third study based on nuclear microsatellites used a smaller sample size because the study was designed for other purposes (Williams et al., 2000). In this study we increased sample sizes to compare *P. taeda* allelic diversity and diagnostic alleles between the eastern and western parts of the species' range. Cross-

validation and independent testing of the results were conducted using a separate set of samples from Mississippi-Alabama.

Methods and materials

Sources of plant material

A sample of 109 *P. taeda* trees represented the periphery of the *P. taeda* range (Fig. 2.1). Seven Mississippi-Alabama samples were used for cross-validation and for an independent test for admixture. These samples, collected from wild stands at ages 30 to 70 years of age ca. 1950-1970, have been conserved through grafting. These archived grafts represent five regions: 29 samples from Maryland, Virginia and North Carolina (NE), 23 samples from South Carolina, Georgia, Florida (SE), 10 samples from northwest of the Mississippi River in Arkansas, Oklahoma, Louisiana and northern Texas (NW), 30 samples from East Texas (SW). Seventeen samples from Lost Pines were analyzed separately then added to the SW region. Geographic distribution of the samples (Fig. 2.1) and estimates of area was estimated using the GIS-based program Arcview 3.2 (ESRI, Redlands, CA).

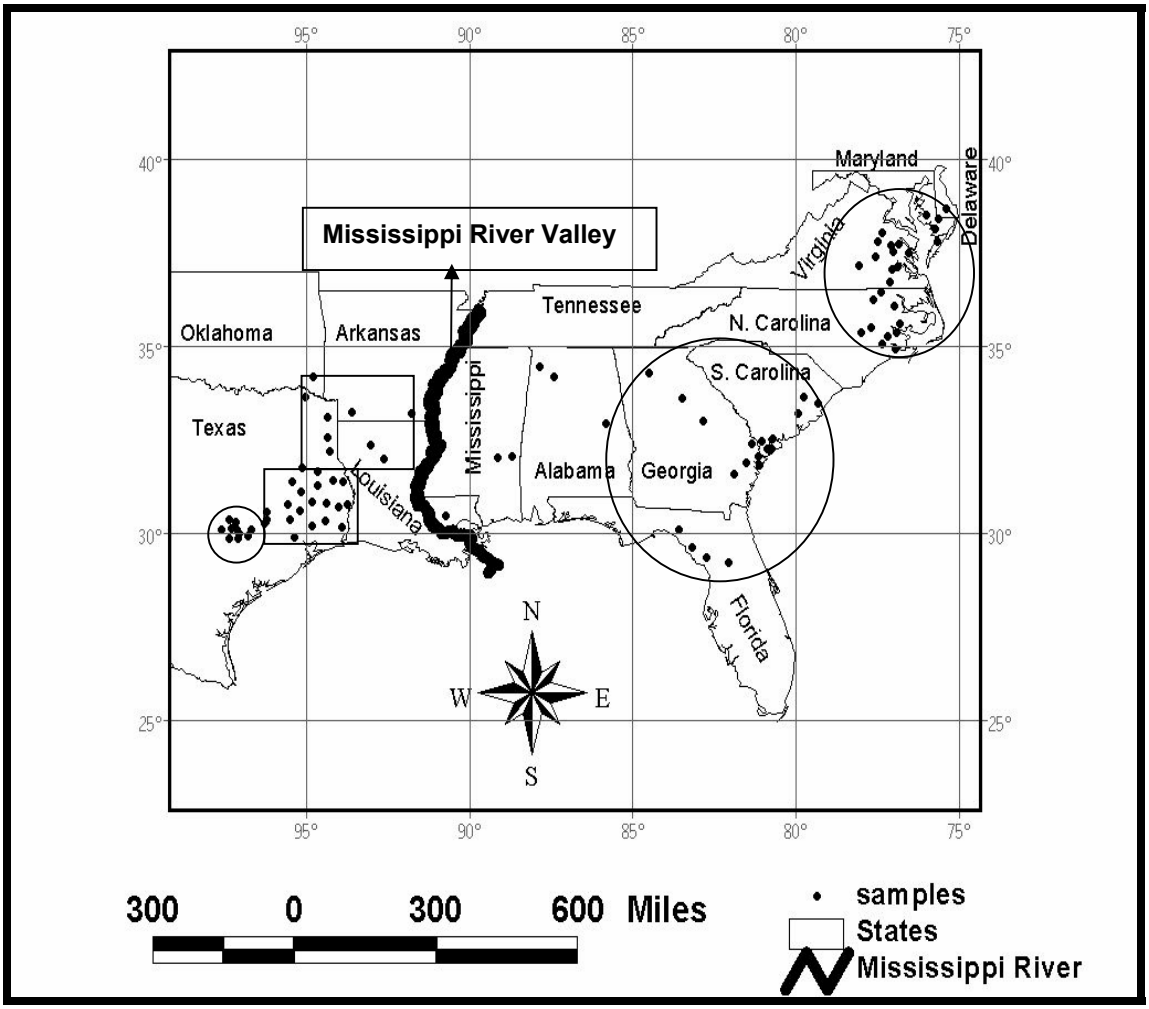

Fig. 2.1. Sampling from five regions within the natural range of *Pinus taeda*: Southeast (SE), Northeast (NE), Northwest (NW), Southwest (SW) and Lost Pines (LP).

DNA isolation and PCR amplification of microsatellites

The DNA extraction of lyophilized needle tissue was based on Doyle and Doyle (1987). The 18 low-copy and genomic microsatellite markers were published in Elsik et al. (2000): PtTX2008, PtTX2037, PtTX2123, PtTX2128, PtTX2142, PtTX2146, PtTX2164, PtTX3011, PtTX3013, PtTX3017, PtTX3019, PtTX3020, PtTX3025, PtTX3026, PtTX3030, PtTX3032, PtTX3034, PtTX3037. GenBank accession numbers for sequences were AF143958 to AF143975. Microsatellite marker protocol was described by Elsik et al. (2000). PCR products were visualized on a silver-stained 6% polyacrylamide denaturing gels. The molecular weight marker pGem (Promega) was used to size alleles.

Statistical analysis

Analyses were conducted using GENEPOP 3.1d (Raymond and Rousset, 1995a) and its Web version (http://wbiomed.curtin.edu.au/genepop/index.html) and BIOSYS-2, a version of BIOSYS-1 (Swofford and Selander, 1981) which was modified for microsatellite analyses by Dr. William C. Black IV, Colorado State University (2/19/99).

Allelic diversity and diagnostic alleles

Allelic frequency, mean number of alleles per locus (MNA), percent polymorphic loci (%P), mean expected heterozygosity (H_e), mean observed heterozygosity (H_o) and diagnostic alleles were estimated for each of the five *P. taeda* regions. H_o was calculated by direct count while H_e was calculated based on Hardy-Weinberg equilibrium. To test for bias in MNA due to unequal sample size or land area distribution, all population samples were randomly adjusted to a uniform size. Five random bootstrapping trials were averaged to give a uniform MNA value. To test for bias in unequal land area distribution between populations east and west of the Mississippi River Valley, an analysis of covariance was conducted using land area as a covariate. Allele frequency was based on direct count and classification was defined as common frequency ($q \geq 0.05$) and rare-frequency ($q < 0.05$) (Marshall and Brown, 1975).

In this study, diagnostic alleles were defined as those which were unique to a region and had frequencies within the common frequency ($q \geq 0.05$) range. Unique alleles in the rare frequency range were defined as potential diagnostic alleles because sampling could not be eliminated as a source of bias.

Genetic differentiation and population substructure

Genetic differentiation was estimated using principal component analysis (Cavalli-Sforza et al., 1994 pp. 39-42) which visually simplifies the large population x allele frequency matrix using orthogonal principal components or factors. The first factor is the compound variable with the largest variance and the second factors, orthogonal to the first, has the next highest variance. A second method, Cavalli-Sforza and Edwards' (1967) chord distance, was estimated for genetic distance among regions. Principal component analysis and chord distances are based on geometric models which do not require assumptions about whether microsatellites mutate by single base substitutions (the infinite alleles model or IAM) or by repeat unit changes (the stepwise mutational model SMM). Principal component analysis (PCA) was based on allele frequencies using PROC FACTOR in SAS 6.12 (SAS Institute, Cary, NC). Standardized scoring coefficients were plotted using Sigmaplot 5.0 (SPSS Inc., 2000). The chord distance (Cavalli-Sforza and Edwards, 1967), assuming pure genetic drift, was calculated as follows:

$$D_c = c\,[1 - \Sigma\,(x, y)^{b}]^{a} \qquad [2.1]$$

Where x and y are frequencies of alleles of size i in populations x and y, respectively and a and b are constants set at 0.5. Constant c equals $2\sqrt{2}/\pi$. The average distance is estimated across all loci.

Population substructure was tested using the Weir and Cockerham (1984) method for estimating Wright's F-statistics where F_{st} is defined as θ. The estimator θ, based on the infinite alleles model (IAM), adjusts for multiple alleles per locus and unequal sample sizes. This is only an approximate measure of population substructure when used with microsatellite data because mutational processes other than IAM may be operative and the contribution of abundant rare-frequency alleles to F_{st} is slight.

Gene flow and the number of migrants

Gene flow estimates were approximated using two methods: 1) by N_m based on θ which tends to overestimate gene flow (Gaggiotti et al., 1999) and 2) by N_m based on the private allele method (Slatkin, 1985) which is more conservative. With smaller sample size per population or region ($n < 50$) and fewer microsatellite loci ($L < 20$), N_m based on θ should perform well despite complex mutational processes (Gaggiotti et al., 1999).

The first method for calculating the effective number of migrants was based on θ. Number of migrants (N_m) per population per generation was calculated as follows (Rossetto et al., 1999):

$$N_m = ¼ ((1/F_{st}) – 1) \quad [2.2]$$

where F_{st} is θ.

For the private allele method, the frequencies of the alleles in a single population are defined as p(l) and private alleles are a function of N_m where a and b are constants that depend on the number of individuals sampled per population:

$$\text{Log } [p(l)] = a \log (N_m) + b \quad [2.3]$$

Results

Genetic differentiation east and west of the Mississippi River Valley was clear from principal components analysis and chord distances. Using three factors, there was separation between the eastern (SE, NE) versus western (LP, SW, NW) parts of the range (Fig. 2.2). The first three factors in the principal components analysis accounted for 95.8% of the total variability. Factors 1, 2

and 3 represented 86.4%, 6.2% and 3.2% of the variability in allele frequencies among populations, respectively. Eigenvalues corresponding to these factors were 4.32, 0.31 and 0.16 (Fig. 2.2) so nearly all of the variability was in the first factor.

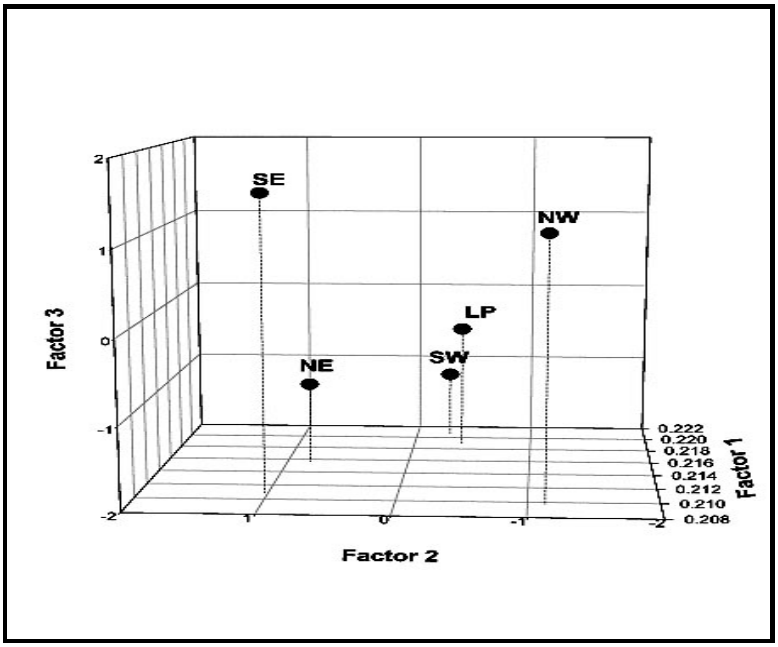

Fig. 2.2. Plot of the first three factors in a principal component analysis based on the allelic frequencies of 18 microsatellite markers scored on the five *Pinus taeda* populations: SE = Southeast, NE=Northeast, NW = Northwest, SW = Southwest and LP = Lost Pines.

The chord distance (D_c) values supported the close relationship between SW and NW regions (D_c = .369) and the more distant relationship between NW and SE regions (D_c = .428) or NW and NE regions (D_c = .414). Similarly, SE was

most closely related to NE (D_c = .315) (Table 2.1). The westernmost part of the *P. taeda* range known as the Lost Pines was closely related to the Southwest region (D_c = .322).

Table 2.1

For five *Pinus taeda* populations, genetic distance was measured using chord distance (D_c) in upper diagonal. Population substructure was estimated using Wright's F_{st} (θ) shown as boldface in lower diagonal.

	NE	**SE**	**SW**	**NW**	**LP**
NE	-	.315	.325	.414	.377
SE	**.0251**	-	.369	.428	.391
SW	**.0264**	**.0473**	-	.378	.322
NW	**.0532**	**.0575**	**.0141**	-	.384
LP	**.0375**	**.0459**	**.0138**	**.0123**	-

Estimation based on F_{st} (θ) values showed most of the genetic variability was within a region or a population rather than among regions. Still, the greatest population differentiation occurred east and west of the Mississippi River Valley as seen by comparing SW to SE (θ = .0473) (Table 2.1). The westernmost Lost Pines population had no apparent separation from SW or NW (θ = .0138, .0123 respectively) (Table 2.1).

Number of alleles and allelic diversity

Allelic diversity was higher for the eastern regions as measured by mean numbers of alleles (MNA). The combined MNA for the eastern *P. taeda* range was 10 compared to 8.7 alleles per locus for the western *P. taeda* range (Table 2.2). Observed heterozygosity was higher in the eastern part of the species' range (0.543 - 0.559) compared to the western range (0.482 - 0.493) (Table 2.2). Observed heterozygosity was lower than expected heterozygosity (Table 2.2).

Table 2.2

Geographic area, sample size, mean number of alleles per locus (unadjusted), mean observed heterozygosity (H_o) by the direct count method and mean expected heterozygosity (H_e) for the five *P. taeda* populations.

Population Structure		Area (km^2)	Sample size	MNA	Mean heterozygosity	
					Observed	Expected
East of Mississippi	Northeast (DE + MD + VA + NC)	75,369	29	8.6 ± 1.4	0.543 ± .055	0.673 ± .056
	Southeast (SC + GA + FL)	152,654	23	8.3 ± 1.2	0.559 ± .061	0.664 ± .067
	Eastern Range Combined	228,023	52	10.0 ± 1.6	0.552 ± .053	0.679 ± .059
West of Mississippi	Northwest (AR+OK+LA+NE-TX)	69,834	10	5.2 ± 0.6	0.482 ± .055	0.639 ± .058
	South West	43,305	30	6.8 ± 0.9	0.493 ± .051	0.660 ± .046
	Lost Pines	26,876	17	6.1 ± 0.7	0.498 ± .047	0.636 ± .047
	Western Range Combined	140,015	57	8.7 ± 1.3	0.493 ± .045	0.656 ± .047
TOTAL		368,038	109	10.8 ± 1.7	0.520 ± .046	0.679 ± .052

Even after adjustment for sample size and land area, *P. taeda* east of the Mississippi River had higher mean allele number compared to the western range (Table 2.3). To test for higher MNA in the eastern range due to unequal sampling, samples were randomly discarded for a uniform comparison. Uniform MNA was still higher for the eastern range after adjustment (10.0 versus 8.5; Table 2.3) so sample size bias did not account for the higher allele number in the eastern part of the *P. taeda* range. Differences in uniform MNA remained statistically significant in the analysis of variance ($p < .0001$; Tables 2.3).

Table 2.3

Uniform mean number of alleles per locus and corresponding observed heterozygosity (direct count) and expected heterozygosity for the five *P. taeda* populations. Sample size was set at ten for each population within the eastern or western *P. taeda* range for uniform size estimation. Uniform sample size was set at N = 51 for the combined analysis.

Population Structure		Uniform MNA	Mean heterozygosity	
			Observed	Expected
East of Mississippi	Northeast (DE + MD + VA + NC)	5.8 ± .76	0.544 ± .060	0.668 ± .059
	Southeast (SC + GA + FL)	6.6 ± .82	0.559 ± .067	0.663 ± .071
	Eastern Range Combined	10.0 ± 1.6	0.552 ± .053	0.679 ± .059
West of Mississippi	Northwest (AR+OK+LA+NE-TX)	5.2 ± .60	0.482 ± .055	0.639 ± .058
	Southwest	4.7 ± .52	0.468 ± .061	0.642 ± .052
	Lost Pines	4.9 ± .50	0.491 ± .052	0.626 ± .052
	Western Range Combined	8.5 ± 1.26	0.491 ± .046	0.653 ± .048

To test for upward bias in MNA due to the larger land area for *P. taeda* east of the Mississippi River, MNA for land area was adjusted using analysis of covariance. There was no significant interaction between land area and location although land area was statistically significant as a covariate. After adjustment for land area and unequal sample sizes, allelic diversity was still higher for the eastern *P. taeda* range (Tables 2.3).

Diagnostic alleles and gene flow

Nine diagnostic alleles and 35 potential diagnostic alleles were found in either east or west of the Mississippi River Valley, making a total of 46 alleles unique to either eastern or western range of *P. taeda* (Table 2.4). Of the nine diagnostic alleles, eight were present in the eastern range and one was present in the western range. The trend towards a higher number of diagnostic alleles in the eastern part of the range was also seen for potential diagnostic alleles: only nine of the 35 rare alleles were unique to the western *P. taeda* range (Table 2.4).

Gene flow was substantial between eastern and western parts of the *P. taeda* range (Table 2.5). The estimated number of migrants (N_m) value was 1.87 to 6.71 migrants per generation per population.

Table 2.4

Nine diagnostic alleles ($q \geq 0.05$) are shown in boldface for *P. taeda* originating west or east of the Mississippi River Valley. Rare-frequency alleles ($q < .05$) were defined as potential diagnostic alleles.

Marker	Allele	Frequency		Marker	Allele	Frequency	
		East	West			East	West
PtTX2037	3	0.020	0.000	PtTX3030	1	0.000	0.046
	5	0.040	0.000		**2**	**0.000**	**0.139**
	6	0.030	0.000		3	0.000	0.046
	9	0.040	0.000		**14**	**0.112**	**0.000**
	10	0.020	0.000		16	0.031	0.000
PtTX2128	2	0.039	0.000	PtTX3032	1	0.010	0.000
	4	0.000	0.020		2	0.030	0.000
PtTX2142	1	0.020	0.000		9	0.000	0.022
	4	0.020	0.000		24	0.010	0.000
PtTX2146	1	0.049	0.000		26	0.010	0.000
	9	0.049	0.000	PtTX3034	10	0.010	0.000
PtTX2164	**10**	**0.150**	**0.000**	PtTX3037	1	0.000	0.010
	14	0.020	0.000		**4**	**0.050**	**0.000**
PtTX3011	2	0.010	0.000		**5**	**0.050**	**0.000**
	3	0.031	0.000		**10**	**0.090**	**0.000**
	5	0.020	0.000		**12**	**0.050**	**0.000**
PtTX3013	3	0.000	0.041		13	0.020	0.000
	4	0.000	0.041		**14**	**0.050**	**0.000**
PtTX3017	3	0.000	0.012		15	0.030	0.000
PtTX3019	1	0.011	0.000		25	0.030	0.000
	9	0.011	0.000		27	0.000	0.010
PtTX3020	1	0.043	0.000		28	0.000	0.010
PtTX3025	10	0.020	0.000		**29**	**0.080**	**0.000**

Table 2.5

Gene flow for *Pinus taeda* L. populations east versus west of the Mississippi River Valley. Gene flow, measured as effective number of migrants per population per generation (N_m), was based on two estimators: F_{st} (defined as θ) and Slatkin's (1985) private allele method.

Estimators	N_m
F_{st}	5.7886
Private allele	1.8684

Validation using the Mississippi-Alabama population

A sample of the Mississippi-Alabama region was used to independently validate diagnostic allele estimates and to test for an east-west *P. taeda* admixture as proposed by Wells et al. (1991). Adding these samples to the original study samples reduced the number of unique alleles only slightly, dropping from 46 to 42. Of these 42 diagnostic alleles, 35 were of eastern origin and seven were from western origin.

The Mississippi-Alabama samples appeared to be part of a continuum or an admixture linking populations east and west of the Mississippi River Valley. Four western alleles were found in the Mississippi-Alabama samples. Similarly, 13 out of 35 diagnostic and potential diagnostic alleles in the eastern range were present in the Mississippi-Alabama samples.

Discussion

There are at least two explanations for the lower allelic diversity in the western range of *P. taeda*: 1) a prevailing westerly wind direction during pollination and 2) western *P. taeda* population contraction over the last several thousands of years coupled with sparser *P. taeda* population density.

Wind direction during the pollination season has been west to east for the past 21,000 years (Bartlein et al., 1998), causing unidirectional gene flow via pollen movement (Wells et al., 1991; Schmidtling et al., 1999). If new alleles arise by mutation in the western part of the range, then these alleles will be transferred via wind-borne pollen across the Mississippi River Valley. These alleles would diffuse through eastern populations, raising allelic diversity of the eastern part of the range without reciprocal exchange. If a new allele arises among the eastern populations, it is more likely to become a diagnostic allele because it is unlikely to be moved westward.

Prevailing west to east wind direction coupled with long-distance viable pollen movement would explain why there are four times as many diagnostic alleles in the eastern part of the range compared to the western range. Similarly, the Mississippi-Alabama region is an eastern part of the species range which has

the highest probability of becoming pollinated by western sources. Thus more of the recent western diagnostic alleles should be found here because there has not been sufficient time for new alleles to diffuse farther east.

A second explanation for lower allelic diversity is gradual population contraction in the westward part of the *P. taeda* range over the past 5,000 to 8,000 years. Evidence from pollen bogs suggests that the range of *P. taeda* was once continuous, extending to the Edwards Plateau in Texas, west of the Lost Pines (Graham and Heimsch, 1960). If so, population contraction due to gradual changes from a cool moist to hot, dry climate in Texas may have reduced population sizes, resulting in recent allele loss (~500 generations or less given 10 years per generation). Similarly, adjustment for mean allele numbers assumes equal population density per unit of land area; density tends to be far sparser in the drier western extremes of the *P. taeda* range and thus lowering the census population size even without range contraction (but see Gram and Sork, 1999).

Number of migrants (N_m) of one or more per generation per population is enough to prevent genetic differentiation between populations (Wright, 1931). Thus gene flow for *P. taeda* is substantial between the contemporary populations east and west of the Mississippi River Valley. Our gene flow estimates were conservative because Mississippi-Alabama samples were

omitted for cross-validation. Previous estimates from an isozyme study which did include the Mississippi-Alabama *P. taeda* population reported higher N_m values ranging 3 to 9 per generation (Schmidtling et al., 1999). Our gene flow estimates were still high enough to preclude genetic drift as the source of the east-west genetic differentiation.

It is paradoxical that gene flow would be high and yet there was still clear genetic differentiation east and west of the Mississippi River Valley. This paradox supports a hypothesis developed by Wells et al. (1991): that genetic differentiation for *P. taeda* is ancient, occurring as a consequence of separation during or preceding the Pleistocene. These ancient, separated populations have expanded to the point that they have recently merged at the Mississippi River Valley. Our results do not support the alternative hypothesis that restricted gene flow caused by the pine-less landform of the Mississippi River Valley itself caused divergence between the eastern and western populations (Florence and Rink, 1979).

The separation of eastern and western *P. taeda* populations was supported by several independent measures of population structure. These estimates should be viewed as conservative due to the possibility of size homoplasy. With size homoplasy, an allele in the eastern range may have the same base-pair length as another allele in the western *P. taeda* range but repeat numbers or flanking

region lengths may be quite different. Size homoplasy problems are accentuated with increasing genetic divergence and are the major drawback to the use of microsatellite bands for phylogenetic inference (Peakall et al., 1998).

Conclusions

Population differentiation was slight throughout the *P. taeda* range but there was clear genetic differentiation east and west of the Mississippi River Valley. *Pinus taeda* east of the Mississippi River had higher allelic diversity than the western part of the range even after adjustment for unequal land areas and sample sizes. Diagnostic alleles were four times more abundant in the eastern part of the *P. taeda* range. Substantial gene flow occurred east and west of the Mississippi River Valley suggesting historical separation of *P. taeda* populations rather than genetic differentiation due to lack of gene flow. Validation of gene flow using Mississippi and Alabama samples supported the gene flow patterns, showing east-west *P. taeda* populations have created an admixture at the juncture of the Mississippi River Valley.

CHAPTER III

RECONSTRUCTING LATE-PLEISTOCENE POPULATION HISTORY OF THE LOST PINES USING MICROSATELLITES

Overview

Late-Pleistocene population history of a disjunct *Pinus taeda* L. population known as the Lost Pines was reconstructed using 34 nuclear microsatellite markers. This population lies in central Texas at the westernmost edge of the species natural range separated by at least 120 kilometers from the larger *P. taeda* population (Western Gulf population) west of the Mississippi River Valley. Results show Lost Pines have undergone at least one bottleneck event. Bottleneck signatures are based on three tests: 1) detection of transient heterozygosity excess which is the difference between expected heterozygosity and heterozygosity at mutation-drift equilibrium; 2) detection of significant reduction in the mean ratio of the number of alleles to the range in allele size (*M*); and 3) detection of the shift in allele frequency distribution (mode-shift indicator which measures when one or more of the intermediate frequency alleles are more abundant than rare frequency alleles). All three tests indicated a bottleneck in the effective population size for the Lost Pines. Shifted allele frequency distribution of the Lost Pines indicated a loss of rare

alleles and a gain of common alleles due to a reduced effective population size (bottleneck). Surprisingly, allelic diversity in the Lost Pines did not decline relative to the control population from the Western Gulf population. Despite the bottleneck, allelic richness was similar for the Lost Pines and the control population: there were 5.29 and 5.38 alleles per locus for the Lost Pines and the control population, respectively. Despite similarity in genetic diversity, only one diagnostic allele in the Lost Pines was absent from the larger Western Gulf population. Twelve diagnostic alleles in the Western Gulf population were absent from the Lost Pines. Explanations for these results, especially the high allelic diversity after a bottleneck event, were discussed. It is proposed that population size expansions and contractions in the Lost Pines parallel historical climate changes in central Texas. These results did not support the steady-state hypothesis that the Lost Pines were constrained to a constant population size by defining soil characteristics.

Introduction

Disjunct populations of species have long fascinated ecologists and evolutionary biologists because they pose critical and interesting questions about the past population dynamics (Whitehead, 1972). Answering such questions given an adequate understanding of a species' response to changing

environmental factors is critical to predicting the future response and fate of such disjunct populations to climate change (Roberts, 1989; Pitelka and The Plant Migration Workshop Group, 1997; Higgins and Richardson, 1999). The rate and magnitude of climatic changes anticipated for the coming century are unprecedented, presenting unique challenges to the biota of the planet (Jackson and Overpeck, 2000). These challenges are especially important for populations facing contraction or extirpation due to unfavorable environmental conditions. Such populations are prone to extinction because of the low rate of gene flow and adaptation and high impact of unfavorable conditions (Davis and Shaw, 2001).

Predicting responses of disjunct populations to changing environmental conditions requires careful reconstruction of the natural history of these populations in the context of past climatic changes. In general, attempts to reconstruct the natural history of organisms in response to climatic change are abundant in the literature (Allnut et al., 1999; Ledig, 1999; Ledig, 2000; Jackson and Booth, 2002; Premoli et al., 2002; Burban and Petit, 2003; Hoffmann et al., 2003; Llewellyn et al., 2003). These studies concentrate on threatened and endangered species because of imminent societal needs. However, the study of disjunct populations for species that are not threatened or endangered is just as important because these studies can elucidate the

consequences of disjunction and isolation. They also can be used as a surrogate for interpreting past climatic changes (Braun, 1955; Axelrod, 1981).

Different approaches are used to reconstruct natural history of populations such as fossil records (pollen data or macro fossils), geological records, morphological phenotypes and molecular markers. The use of DNA markers, especially microsatellites, and the analysis of their genetic variation among contemporary individuals had proven powerful in reconstructing natural history of different species (Jarne and Lagoda, 1996; Goldstein and Pollock, 1997; Beaumont and Bruford, 1999; Linares, 1999; Barbujani and Bertorelle, 2001; Waldick et al., 2002; Cavalli-Sforza and Feldman, 2003). A population's response to changing climatic conditions can leave a significant genetic imprint. The genetic imprint or DNA signature following a population contraction or a bottleneck has been the focus of theoretical and empirical studies alike (Nei et al., 1975; Maruyama and Fuerst, 1985a, 1985b; Cornuet and Luikart, 1996; Garza and Williamson, 2001). These changes are far from being understood in natural forest tree populations.

The Lost Pines population is a good candidate ecosystem for investigating the genetic consequences of population contraction. Its population may have shifted in response to extreme climate change in central Texas over the past 21,000 years or more. This is a disjunct *Pinus taeda* L. population at the

westernmost edge of a colonizing coniferous species which has present-day range throughout the southern quadrant of the United States (Fig. 3.1). A set of late-Pleistocene hypotheses can be developed for the Lost Pines given life history attributes as well as prehistoric, paleoclimatic and fossil records. These hypotheses can then be tested using DNA markers.

Natural range and life history of Pinus taeda

The present-day species range starts in Delaware, goes southward to Florida then continues westward to Texas. The Lost Pines marks the westernmost edge of the species' range (Fig. 3.1). *Pinus taeda* is a long-lived woody perennial which aggressively colonizes open areas early in succession through heavy annual seed production. Generation intervals are approximately 15 years per generation (Dorman, 1976), but longer generation intervals of 15 to 30 years are assumed because the generation interval for *P. taeda* tends to be longer in fire-prone areas. If a fire occurs in less than 10 years or more than 30 years, *P. taeda* seedlings are not likely to survive (Pyne et al., 1996), which effectively lengthens time interval between successive generations. Pre-settlement fire history in east Texas shows that major fires occurred in a cycle of less than 35 years (Wade et al., 2000).

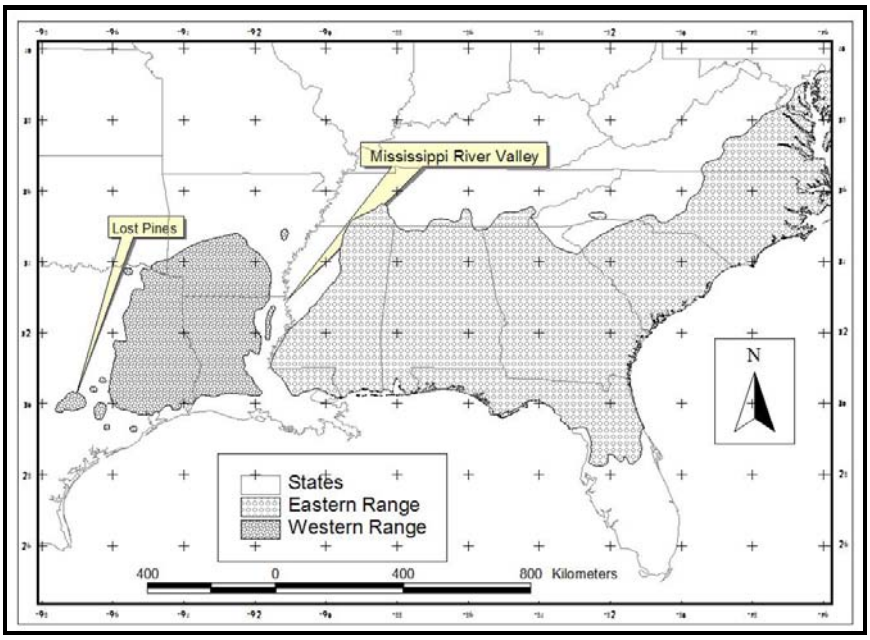

Fig. 3.1. A layout of the Lost Pines relative to the natural range of *P. taeda.* The natural range is divided by the Mississippi River Valley (modified after Critchfield and Little, 1966).

The Lost Pines population appears to be a natural occurrence (see Chapter IV). Spanish explorers, Anglo-American settlers, naturalists and geologists reported seeing pure stands of pines in central Texas (Buckley, 1866; Kennedy, 1925; Anonymous, 1952; Fitzwilliam, 1955; Youngman, 1965). Based on genetic marker analysis, the Lost Pines population is closely related to east Texas *P. taeda* populations (Florence and Rink, 1979; Williams et al., 2000; Al-Rabab'ah and Williams, 2002).

The Mississippi River Valley (MRV) separates between the eastern and western range of *P. taeda* (Critchfield and Little, 1966; Baker and Langdon, 1990, Fig. 3.1) which are genetically differentiated from each other (Florence and Rink, 1979; Wells et al., 1991; Al-Rabab'ah and Williams, 2002). High levels of genetic diversity are maintained through outcrossing and long-distance gene flow via pollen movement reaching over 600 km in the planetary boundary layer 10 km above ground (Bessey, 1883; DiGiovanni et al., 1996). Prevailing west to east jet stream winds over the past 21,000 years (Toomey et al., 1993; Bartlein et al., 1998) are hypothesized to cause the higher genetic diversity and private alleles in *P. taeda* populations east of the MRV.

Fossil records and Holocene climate change in central Texas

Palynological analysis shows that the climate transition in central Texas moved gradually from cool-moist to warm-dry climate with periodic fluctuation between dry and moist conditions (Graham and Heimsch, 1960; Patty, 1968; Bryant, 1977; Bryant and Holloway, 1985). A Holocene climate shift is evident from river hydrology, cave formations and radioisotope studies (Toomey et al., 1993, Musgrove et al., 2001). The climate shift includes prolonged periods of drought and unusually high temperatures, higher than contemporary temperatures, defined as an Altithermal or Hypsithermal (Deevey and Flint, 1957; Wells,

1970; Bryant, 1977; Davis, 1984; Toomey et al., 1993; Waters and Nordt, 1995; Bousman, 1998; Wilkins and Currey, 1999; Hester and Turner, 2003).

Two hypotheses have been proposed to explain the disjunct nature of the Lost Pines. The population contraction hypothesis proposes that the pine forest in central Texas could have been more extensive during the late-Pleistocene owing to a milder climate and more adequate moisture and that the pine forest later contracted with Holocene climate change. Support for this hypothesis comes from paleosoils studies (Sorenson et al., 1976; Toomey et al., 1993)

During the late-Pleistocene, the pine forest is hypothesized to have expanded to the west of its present range. Expansion may have been confined to terraces along the Colorado River towards the Edwards Plateau (Sorenson et al., 1976), a suture zone where boreal, temperate and subtropical flora and fauna are believed to have co-existed (Remington, 1968). By early-Holocene, climate starts to gradually change to warmer and drier conditions. Continued harsh climate changes and prolonged drought in central Texas during the mid- to late-Holocene (Toomey et al., 1993; Russ et al., 2000) are hypothesized to have caused *P. taeda* populations to retreat to the present-day range around Bastrop State Park. The Lost Pines population size may have been even smaller than contemporary population size since temperatures and

precipitation regimes were more severe then than present-day regimes (Toomey et al., 1993).

The steady-state hypothesis provides an opposing view. This hypothesis proposes that there has been neither expansion nor contraction of the Lost Pines population. Support for this hypothesis comes from current soil mapping. The boundaries of this disjunct pine forest may be set by the existence of a certain soil type, mainly sandy or sandy-loam surface soils underlain by heavier sandy-clay soils (McBryde, 1933). If so, the Lost Pines population size would have been at the same size for millennia (Silker, 1973). This hypothesis was supported by one palynological study which implies that the concentration of pines in central Texas has been high for 12,000 years (Patty, 1968 pp. 40-41).

A test for bottleneck signatures should discern between these two hypotheses. Under the population contraction hypothesis, contractions in the effective population size (N_e) defined as a genetic bottleneck should be detected. These contractions would be restricted to *P. taeda* in central Texas where Holocene droughts were most extreme. Under the steady-state hypothesis, no population bottleneck is predicted for the Lost Pines because the population size has been large enough to prevent a genetic bottleneck.

Detecting genetic bottlenecks

In theory, peripheral populations undergoing genetic bottleneck should have decreased allelic diversity (Nei et al., 1975; Maruyama and Fuerst, 1985a, 1985b) due to loss of rare alleles. To detect population bottlenecks, estimates of the population size before and after the bottleneck are ideally needed. Or, bottleneck can be inferred if a loss of allelic diversity in a population was detected, which also requires sampling the population before and after the bottleneck or sampling another population as a control. These requirements are not always met in practical situations.

A recent advance in bottleneck theory shows that genetic bottlenecks can be detected without an estimate of the original population size or a temporal control (Spencer et al., 2000). This requires detection of excess heterozygosity (Cornuet and Luikart, 1996) or reduction on *M*, the mean ratio of the number of alleles to the range in allele size (Garza and Williamson, 2001). Use of these tests circumvents some historical problems associated with detecting bottleneck events using genetic diversity measures alone (Matocq and Villablanca, 2001). Losing rare alleles can be also detected if one or more of common allele classes have a higher number of alleles than the rare allele class in a population. This is known as the mode-shift indicator (Luikart et al., 1998).

Excess heterozygosity tests detect bottleneck events over a time span of $0.2N_e$ to $4N_e$ generations (Cornuet and Luikart, 1996); however, the mode-shift indicator can detect bottleneck over a time span of only $2N_e$ to $4N_e$ generations (Luikart et al., 1998) narrowing the time span for the bottleneck. If generation time ranges from 15 to 30 years and the bottleneck N_e values range from 50 to 1000, then this time interval excludes the 19th century logging history as the cause of the bottleneck event if detected. Even so, the Lost Pines population size was estimated to be nearly the same between the mid 19th century and the mid 20th century (Easton, 1947; Anonymous, 1952). Up until the mid 20th century, logging companies were able to produce sustainable amounts of lumber from the Lost Pines area each year (Easton, 1947).

The study objective is to discern between the two hypotheses accounting for present-day disjunct distribution of the Lost Pines population. The population contraction hypothesis predicts that 1) bottleneck signatures should be present in the Lost Pines population and 2) allelic diversity should be lower for the Lost Pines population relative to its control. If the steady-state hypothesis is true, then there should be no bottleneck signature, but allelic diversity may be lower than the control due to partial or full reproduction isolation.

Methods and materials

Population sampling

All samples in this study were collected from wild stands in 1952 to 1970 at ages 30 to 100 years then conserved in grafted archives for *ex situ* conservation. The archives represent wild *P. taeda* populations prior to domestication. A total of 78 samples were collected as follows: 60 samples from the populations west of the Mississippi River Valley or the Western Gulf (WG) region and 18 samples from the Lost Pines in Bastrop (14 samples) and Fayette (4 samples) Counties in central Texas (Fig. 3.2). The WG samples include 14 samples from Polk County, Texas, which served as the control population.

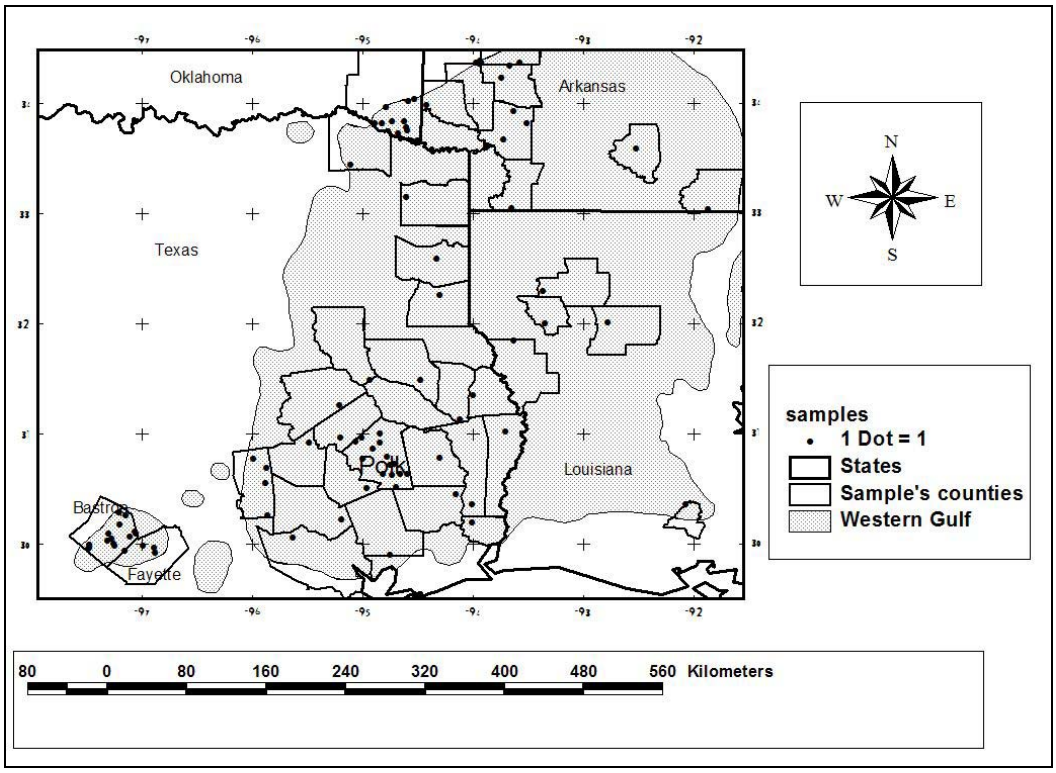

Fig. 3.2. A layout of the Lost Pines counties relative to the Western Gulf counties. Each dot in a county represents a sample from the county with no reference to its exact location within the county.

The 18 samples from the Lost Pines are used to test for bottleneck. The 14 samples from the Lost Pines (Bastrop) and the equivalent 14 samples from the control (Polk) are used to compare genetic diversity. The 60 samples from the WG region and the 18 samples from the Lost Pines are used to determine diagnostic alleles because this analysis should include all available samples.

Nuclear microsatellite markers

Lyophilized needle tissue protocol for DNA extraction was based on the approach of Doyle and Doyle (1987). A set of 34 microsatellite markers were randomly sampled from a list of 245 genomic (G), low copy (LC) or undermethylated (UM) microsatellites (Auckland et al., 2002): PtTX2008, PtTX2037, PtTX2080, PtTX2082, PtTX2093, PtTX2094, PtTX2123, PtTX2128, PtTX2142, PtTX2146, PtTX2164, PtTX3011, PtTX3013, PtTX3017, PtTX3019, PtTX3020, PtTX3025, PtTX3030, PtTX3032, PtTX3034, PtTX3026, PtTX3037, PtTX3118, PtTX4001, PtTX4003, PtTX4009, PtTX4011, PtTX4036, PtTX4046, PtTX4056, PtTX4062, PtTX4093, PtTX4100, PtTX4112. Detailed lab protocols, primer annotation and primer sets are provided in Auckland et al. (2002). PCR products were visualized on silver-stained 6% polyacrylamide denaturing gels. The sizes of the alleles were determined using the pGem molecular ladder (Promega).

Statistical analysis

The following analyses were performed using GENEPOP 3.3 (Raymond and Rousset, 1995a). Deviations from Hardy-Weinberg equilibrium (HWE) were estimated to test for bias in bottlenecks due to deviations from HWE. Deviations were based on Fisher's exact tests (χ^2) (Raymond and Rousset,

1995b). In addition to the exact test, a Markov-chain algorithm was used for loci with five or more alleles to obtain an unbiased estimate of the exact probability of incorrectly rejecting HWE (Rousset and Raymond, 1995). In all cases, the Markov chain was set to 10000 steps of dememorization, 10000 iterations per batch and 200 patches.

Bottleneck events were tested using three methods. The first method is called the excess heterozygosity test and it was developed by Cornuet and Luikart (1996). This method can be conducted using three independent tests. The sign test, standardized difference test and Wilcoxon's signed rank test were used to detect bottleneck using the software package called BOTTLENECK v. 1.2.02 (Piry et al., 1999). The sample size of 18 is close to the sample size required for the test (20); However, power of the test can be improved by scoring >20 polymorphic microsatellite markers (Cornuet and Luikart, 1996).

For a population at mutation-drift equilibrium, expected heterozygosity (H_e) and heterozygosity under mutation-drift equilibrium (H_{eq}) are equal. Consequently, the ratio between them (H_e / H_{eq}) should be one if no bottleneck has occurred. For a population undergoing a bottleneck and losing rare alleles, H_e will be in excess of H_{eq} (Cornuet and Luikart, 1996), thus their ratio will be in excess of one. H_e is more sensitive to allele frequencies rather than number of alleles (Nei, 1987). Hence, when a population loses rare alleles, H_e declines slightly.

Conversely, H_{eq} is more sensitive to the number of alleles rather than allele frequency. Hence, when a population loses rare alleles, H_{eq} decreases more than H_e does.

Simulation of H_{eq} distributions assumed the two-phase mutation model (TPM) (DiRienzo et al., 1994) with 70% single-step mutations and 30% of multiple step mutation and a variance of 30 which approximates the mutation model of the markers used in this study. Results of the simulation based on the stepwise mutation model (SMM) and infinite allele model (IAM) were also reported as confidence intervals.

The second method is the graphical representation of the mode-shift indicator originally proposed by Luikart et al. (1998). Losing rare alleles in bottlenecked populations will be also detected when one or more common allele classes have a higher number of alleles than the rare allele class (Luikart et al., 1998). This test was re-scaled so that frequency distribution of the allele frequency classes in each population would be based on equal 0.05 increments. Sample size of 18 is low for this particular test compared to the requisite 30 samples (Luikart et al., 1998). However, these samples can provide some insight into the data.

The third method is called the *M* ratio test (Garza and Williamson, 2001). The test was conducted using a program called M_P_Val.exe. Sample size (18) was lower than recommended for the test (25), but number of loci used (18) is much higher than the minimum (5) loci suggested for this test (Garza and Williamson, 2001). Not all 34 *P. taeda* microsatellite loci could be used in this test. Number of sampled individuals are assumed to be at least twice the number of alleles at the most variable marker, thus only 18 markers with number of alleles of 9 or less could be used.

The ratio of the number of alleles (*k*) at a locus to the range of allele sizes for the same locus (*r*) is called the *M* ratio (Garza and Williamson, 2001). The *M* ratio should be stable if the population size was stable for a long time regardless of the population size. When a population experiences a bottleneck event, alleles will be lost and *k* will be reduced. However, lost alleles are not necessarily at the extremes of the allele size distribution so the range in allele size (*r*) will not be reduced at the same rate as *k*. Consequently *M* ratio will drop in the event of a bottleneck event. To test whether a value for *M* is lower than expected or not, 10,000 replicates were simulated. The number of times that the simulated *M* is higher than the calculated *M* represents the statistical significance of the *M*-value reduction.

Simulation of *M* requires few parameters. Some of the parameters are not known so a sensitivity analysis was conducted to bracket the possible ranges of *M* values. The first parameter is called theta (θ). Theta equals:

$$\theta = 4 \times N_e \times \mu \qquad [3.1]$$

where

N_e is the effective population size before the bottleneck
μ is the mutation rate, assumed to be 0.001.

N_e was assigned the values of 100, 500, 1000, 5000 ranging from being small all the time (N_e = 100) all the way to being large (N_e = 5000). Accordingly, values of θ were 0.4, 2, 4, and 20.

The second parameter in the *M* test is called the average multi-step mutation. Assuming that a mutation is not always a single-step, there will be multi-step mutations which will change the microsatellite length by more than one repeat. These multi-step mutations may vary from 2 to 3 or more repeats or steps. On average, these mutation were assumed to change the microsatellite length by 2, 2.8, 3 and 3.5 steps bracketing the 2.88 value reported by Garza and Williamson (2001) in their literature survey. The value 3.5 is considered to be

very conservative (Garza and Williamson, 2001). The probability of these multi-step mutations was set to 30%, the same value used in the excess heterozygosity test mentioned earlier.

Genetic diversity measures for the Lost Pines were compared to the Western Gulf population using Polk County samples. The geographical area for Polk County is similar to that of Bastrop County (2800 km^2 versus 2330 km^2, respectively, based on estimates from ARCVIEW 3.2 (ESRI, Redland CA)). Although population densities differ, no bias in allelic diversity is expected (Gram and Sork, 1999). Mean number of alleles per locus, mean expected heterozygosity (H_e), mean observed heterozygosity (H_o), mean heterozygosity under mutation-drift equilibrium (H_{eq}), and percent polymorphism (%P) were calculated using the software package GENETIX v. 4.02 (Belkhir et al., 2002). All diversity measures were tested for significant differences using a t-test.

Private alleles are alleles present in one population and absent from the other (Slatkin, 1985). Diagnostic alleles are defined here as private alleles if they are present in one population with a frequency of more than 10% and absent from another. Diagnostic alleles provided a more stringent criterion because chances of confounding allele classifications with sampling errors were decreased. Comparisons for private and diagnostic alleles were made between the 18 Lost Pines samples (LP_{18}) and the 60 Western Gulf samples.

Results

One or more genetic bottleneck events were detected in the Lost Pines. Heterozygosity excess, reduced *M* ratio and shifted allele frequency distribution were all detected in samples of the Lost Pines. Allelic diversity measures were similar for the Lost Pines and its control, but private and diagnostic alleles were higher in the control population.

Heterozygosity excess was statistically significant for the Lost Pines samples under the assumed mutation model (TPM). Results were similar for the sign test, standardized difference test and Wilcoxon's signed rank test. These tests were significant for LP_{18}; p-values were 0.050, 0.031 and 0.015 levels respectively (Table 3.1). None of the three tests was significant for Polk County at the 5% level for TPM; p-values were 0.201, 0.335 and 0.117 respectively (Table 3.1). Results of the IAM and SMM showed values that bound the TPM values but these mutational models do not represent the appropriate mutational model for the nuclear microsatellites. IAM results were significant for the Lost Pines samples but not for Polk samples. Results of the pure SMM revealed no significance for both the Lost Pines and Polk County; however, the p-values for the Lost Pines are lower than those for Polk.

Table 3.1

A bottleneck was detected for the Lost Pines. The occurrence of a bottleneck event was tested using three statistical tests: the sign test, the standardized difference test and Wilcoxon's signed rank test. Results for the three mutation models are reported with the bold numbers referring to the assumed TPM. Tests results for the control population were also reported. DEF refers to tests that have detected heterozygosity deficiency instead of excess.

	Mutation Model	Statistical significance levels	
		Lost Pines (LP_{18})	Polk County
Sample size (N)		18	14
Sign test	IAM	0.005	0.063
	TPM	**0.050**	**0.201**
	SMM	0.112	0.381
Standardized difference test	IAM	0.000	0.096
	TPM	**0.031**	**0.335**
	SMM	DEF	DEF
Wilcoxon's signed rank test	IAM	0.000	0.071
	TPM	**0.015**	**0.117**
	SMM	0.091	0.694

A genetic bottleneck was also evident from the mode-shift indicator. Allelic distribution approximated the expected normal L-shape for Polk County, but allelic distribution shifted for the Lost Pines due to loss of rare alleles (Fig. 3.3). For the Lost Pines, the proportion of rare alleles ($q < 0.05$) was 19.9%, but the proportion of the next allele class ($0.05 < q < 0.10$) was higher at 24%.

Significant reduction of *M* was also detected for the Lost Pines over wide ranges of assumed parameters (Table 3.2). *M* value for the Lost Pines was 0.511, significantly lower than the expected value regardless of the wide ranges of assumed values of θ and the average multi-step mutations (Table 3.2).

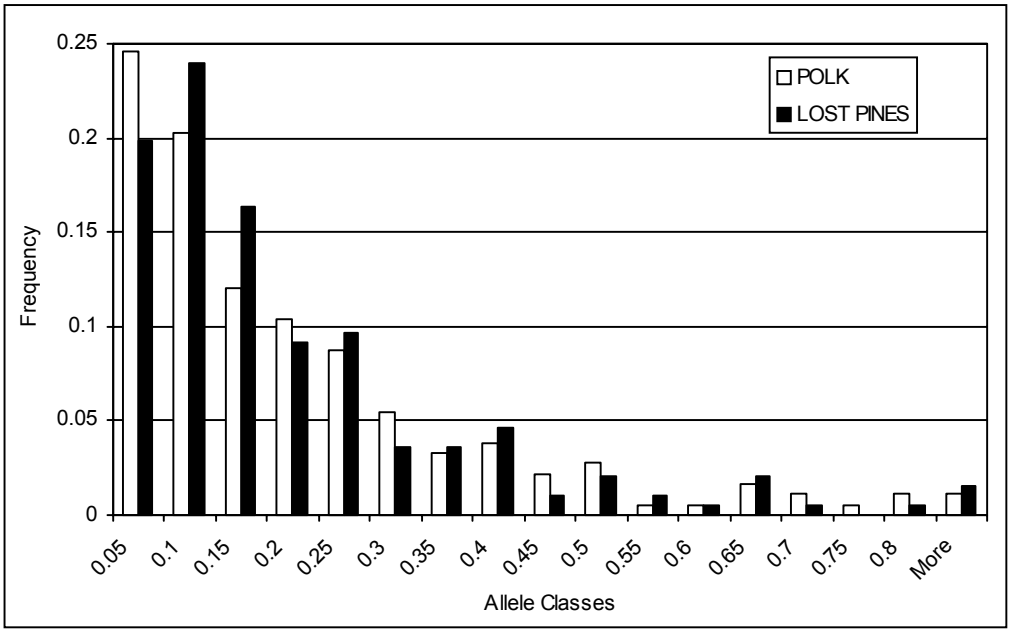

Fig. 3.3. Frequency distribution of allele classes for the Lost Pines compared to Polk as a graphical representation of the mode-shift indicator. The Lost Pines frequency distribution is shifted indicating reduction in the effective population size.

Table 3.2

A bottleneck was detected for the Lost Pines. The occurrence of a bottleneck event was detected as a reduction of the *M* ratio (the ratio of allele numbers to the range in allele size). Results show the significance of the reduction in *M* ratio (*M* = 0.511) based on simulations of four different values of θ, four different values for multi-step mutation scenarios, 18 microsatellite markers, mutation rate of 0.001 and the TPM mutation model with 30% multiple-step mutations.

Average multi-step mutations	θ			
	0.4	2.0	4.0	20
2.0	0.0000	0.0000	0.0000	0.0000
2.8	0.0000	0.0000	0.0000	0.0001
3.0	0.0000	0.0000	0.0000	0.0013
3.5	0.0000	0.0001	0.0021	0.0091

Allelic diversity measures were similar for the Lost Pines and its control population from the WG population (Table 3.3). The 34 microsatellites were 100% polymorphic in all samples. Mean number of alleles (MNA) was nearly identical for the LP_{14} (5.29) and for Polk County (5.38) with no significant difference revealed by the t-test (p-value < 0.38). Heterozygosity measures were also similar for the Lost Pines and its control (Table 3.3). To reduce the effect of apparent skewness in the distribution of allele frequency, Wilcoxon's rank test was used. There were no significant differences between the Lost Pines and its Polk County control (Table 3.3).

Table 3.3

Genetic diversity was similar for the Lost Pines (LP) and the large continuous *Pinus taeda* population west of the Mississippi River Valley represented by Polk County, Texas. Sample size, percent polymorphism, mean number of allele per locus (MNA), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e) and unbiased mean expected heterozygosity (H_{eq}) are shown with their standard error of estimates (in parentheses). The t-test was not significant for any of these measures at the 0.05 level of statistical significance.

Population	LP	Polk	T-test
Sample size	14	14	
Total number of alleles	180	183	
Percent Polymorphic loci	100	100	
Mean number of allele/locus (MNA)	5.29 (0.43)	5.38 (0.40)	0.38
Allelic Richness (Random sample size of 10)	4.9 (0.37)	5.1 (0.37)	0.27
Mean observed heterozygosity (H_o)	0.46 (0.04)	0.48 (0.04)	0.28
Mean expected heterozygosity (H_e)	0.65 (0.03)	0.64 (0.03)	0.45
Unbiased mean expected heterozygosity (H_{eq})	0.67 (0.03)	0.67 (0.03)	0.45

There were few private or diagnostic alleles for the Lost Pines compared to the Western Gulf (Table 3.4). Out of 314 total alleles, only three private alleles were detected for the Lost Pines population. One allele was diagnostic but sampling error could not be ruled out (Table 3.4). By comparison, Western Gulf had a total of 28 private alleles and 12 of the 28 were diagnostic ($q > 0.10$).

Table 3.4

Diagnostic alleles ($q \geq 0.10$) for Lost Pines and Western Gulf range of *Pinus taeda* populations. Bold number shows the only unique allele to the Lost Pines. Total private alleles ($q > 0.05$) are also reported.

Marker	Allele Number (bp)	Allele Frequency	
		Lost Pines (allele copies)	Western Gulf (allele copies)
PtTX2008	316	0.00	0.18 (19)
PtTX2082	227	0.00	0.17 (17)
PtTX2164	269	0.00	0.12 (13)
	248	0.00	0.10 (10)
PtTX3032	**308**	**0.10 (3)**	**0.00**
PtTX3034	194	0.00	0.10 (11)
PtTX3037	167	0.00	0.10 (10)
	165	0.00	0.12 (12)
PtTX4001	223	0.00	0.16 (18)
	220	0.00	0.13 (15)
PtTX4009	288	0.00	0.11 (12)
PtTX4046	242	0.00	0.21 (23)
PtTX4056	457	0.00	0.12 (13)
Total diagnostic		1	12
Total private		3	28

Discussion

The population contraction hypothesis was better supported than the steady-state hypothesis. The Lost Pines populations have had dynamic population size shifts (expansion and contraction) which paralleled the climatic history of

central Texas. The cause for high genetic diversity despite one or more significant bottleneck events is yet to be determined. Private and diagnostic allele distribution supported earlier observation of unidirectional west-to-east pollen and gene flow. Environmental conditions appear to have shaped where the Lost Pines population persists today but this aggressive colonizing species is adapted broadly enough that population expansion was possible over a wider range of soil types.

The Lost Pines population had few private alleles and one diagnostic allele; all other alleles were shared with the Western Gulf population with one exception. PtTX3032 allele 308 occurred with three allele copies. Given the small sample sizes, this could be attributed to sampling error. One explanation is that west-to-east unidirectional movement of alleles reported across *P. taeda* range (Al-Rabab'ah and Williams, 2002) was mirrored in a smaller scale in this study. Conversely, Western Gulf population had 28 private alleles and 12 diagnostic alleles which were not found in the Lost Pines population. One explanation is that novel mutations in the Lost Pines population move eastward on prevailing winds without reciprocal exchange. Gene flow from the Lost Pines to the western part of the *P. taeda* range is most likely to occur as a result of long-distance pollen dispersal.

Lost Pines population size parallels the paleo-ecological records

The paleo-ecological record shows fluctuated climate cycles in central Texas. This fluctuation consisted of alternating xeric and mesic conditions throughout the late-Pleistocene especially during the Holocene (Davis, 1984). The LGM was characterized by cooler temperature as much as 4 to 6°C or more with increased moisture (Graham and Heimsch, 1960; Barry, 1983; Holloway and Bryant, 1984; Bryant and Holloway, 1985; Toomey et al., 1993; Waters and Nordt, 1995; Musgrove et al., 2001). The winter temperatures may have not been considerably cooler than the present-day winter temperatures (Holloway and Bryant, 1984; Bryant and Holloway, 1985; Stute et al., 1992; Toomey et al., 1993). These conditions could have ameliorated the dry central Texas climate to allow westward expansion (Sorenson et al., 1976). Mesic conditions persisted through the Late-Glacial (14,000-10,000 YBP) with temperatures 2 to 3°C cooler than present (Toomey et al., 1993).

The onset of the Holocene as a warm period (Porter, 1983; Mickelson et al., 1983) is believed to have invoked population size contraction for the Lost Pines. In central and south-central Texas, the warming drying trend is believed to have culminated during the Mid- to Late-Holocene (8,000-2,500 YBP) in what is called the Altithermal or the Hypsithermal (Deevey and Flint, 1957; Wells, 1970; Bryant, 1977; Davis, 1984; Toomey et al., 1993; Waters and

Nordt, 1995; Bousman, 1998; Brown, 1998; Goodfriend and Ellis, 2000). This change to a warmer climate may have caused the westernmost *P. taeda* populations become the retreating edge of the species' range.

For example, there was a reduction in fossil pine pollen quantities during the onset of the Holocene around 8000 YBP which reached minimum during the peak of the Altithermal conditions ca. 5000–2500 YBP (Bryant, 1977; Bryant and Holloway, 1985; Toomey et al., 1993). The fluctuation between mesic and xeric climatic conditions may have happened multiple times during the Holocene (Davis, 1984; Russ et al., 2000). If the warm-temperate pollen is indeed from local *P. taeda* forests, then population history of the Lost Pines may have paralleled these cycles with a series of bottleneck and expansion events until now. The drawback of the pine pollen record is the lack of their affinity to the species level; however, pine pollen in central Texas is believed to belong to either one of the three prevalent southern pine species; *P. taeda*, *P. echinata* and *P. palustris*.

High allelic diversity levels despite a bottleneck event

The study results contradicted the theoretical prediction of reduced allelic diversity following a bottleneck (Nei et al., 1975; Maruyama and Fuerst, 1985a, 1985b; Leberg, 1992; Spencer et al., 2000; Garza and Williamson, 2001;

Whitehouse and Harley, 2001). Few studies have documented a bottleneck event where allelic diversity was not reduced (Hartl and Hell, 1994; Luikart, 1997; Keller et al., 2001). The following is a discussion of the possible causes for the observed high allelic diversity despite the bottleneck event.

One likely explanation for the high allelic diversity in the Lost Pines is that the bottleneck event was followed by expansion during favorable conditions. Bottleneck signatures can persist long time and require long time to disappear due to population recovery (Garza and Williamson, 2001) but genetic diversity can recover faster with favorable environmental conditions. If the bottleneck was assumed to have happened during the past few hundreds of generations or more, signatures of bottleneck would still be detected in present-day Lost Pines population despite any period of population recovery. However if there were periods of recovery and expansion, genetic diversity will recover fast.

A less likely explanation is increased migration via pollen from local sources. The bottleneck events may have caused pine populations in central Texas to become more fragmented and less dense, which in turn increased migration rates. Reduced stand density and increased fragmentation in shortleaf (*Pinus echinata*) increase allelic diversity of pollen pool because pollen migrates more readily from nearby sources (Dyer and Sork, 2001). Closed and dense forests filter outside pollen but open forests or single trees permit more outside pollen

migration (Comps et al., 2001; Dyer and Sork, 2001; Widmer and Lexer, 2001). This explanation is consistent with the experimental observations that low levels of migration increase allelic diversity in bottlenecked populations (Keller et al., 2001; Ebert et al., 2002). The only drawback to this explanation is that it violates the assumption of no migration, a basic tenet of mutation-drift equilibrium and the heterozygosity excess method (Cornuet and Luikart, 1996). The degree to which pollen migration can actually generate heterozygosity excess and falsely indicate bottleneck events has not been determined.

A third explanation is that pre-bottleneck allelic diversity levels in the Lost Pines were initially higher. This implies that Polk County is not an adequate substitute for the original Lost Pines population prior to the bottleneck event(s) and thus does not approximate true temporal control. If so, the original population would have had more alleles per locus than present. Pre-bottleneck genetic diversity for the Lost Pines is unknown but can be roughly estimated using simulation studies. However, the highest observed genetic diversity throughout the present-day *P. taeda* range is 10 alleles/locus (Al-Rabab'ah and Williams, 2002). Whether a pre-bottleneck genetic diversity of 10 alleles/locus decreasing to 5.29 alleles/locus after a bottleneck event is enough to generate heterozygosity excess has not been determined.

Balancing selection due to heterozygote advantage (Carson, 1990; Bancroft et al., 1995) is not a likely explanation for high allelic diversity in the Lost Pines. QTL analyses of these marker loci infer that only a few have overdominant gene action in selfed pedigrees (Williams et al., 2001). Similarly, retention of hidden allelic diversity by seed banks either via serotinous cones or by long-term seed viability in forest soil layers (Levin, 1990; McCue and Holtsford, 1998; Tapias et al., 2001) is not likely because *P. taeda* does not have serotinous cones or long-lived seeds (Baker and Langdon, 1990; Shelton and Cain, 2001).

Timing of the bottleneck: a proposed model

Significant heterozygosity excess and the mode-shift indicator place the time of the bottleneck within the past 2 to $4N_e$ generations (Cornuet and Luikart, 1996; Luikart et al., 1998). Assuming a generation interval of 15 to 30 years and population size of 50 to 1,000 places the bottleneck event between 1,500 YBP and 120,000 YBP. The significant reduction of the *M* ratio places the bottleneck event a shorter timeframe of 1,000 generations or 30,000 YBP (Garza and Williamson, 2001).

Aligning climatic events in this wide timeframe yielded some valuable insights. During the past 120,000 years, two interglacial periods followed two glacial

periods. The first glacial period occurred between 120,000 YBP and 100,000 YBP (Emiliani, 1971; Barnola et al., 1987). This glacial period was followed by another interglacial period between 100,000 YBP and 70,000 YBP (Emiliani, 1971; Barnola et al., 1987). The last glacial period started 70,000 YBP and ended 11,000 YBP and reached the peak (LGM) around 20,000 YBP (Emiliani, 1971; Barnola et al., 1987). The current interglacial period started 11,000 YBP.

The current genetic bottleneck could have occurred within the last interglacial (70,000 – 100,000 YBP) or within the current interglacial (the past 11,000 YBP), or within both interglacial periods. If a bottleneck event happened during the last interglacial period followed by a period of approximately 60,000 years of glaciation (70,000 YBP minus 11,000 YBP), these approximately 2,000 to 4,000 generations might have been enough to mask the first bottleneck changes. This suggests that current bottleneck signatures must belong to the current interglacial with some faint signatures of a bottleneck event from the last interglacial period.

Expansions and reductions in the Lost Pines population size are proposed to be concomitant with the current interglacial period, a parallel to changes in moisture and temperatures before and during the Holocene (Table 3.5). Under this proposed scenario, a series of population expansions may have occurred in the cool-moist climate. Population expansions would have been followed by

a slight reduction or a bottleneck event around 8,000 YBP. Other sharper bottleneck events may have occurred during the peak of the Altithermal 5,000 to 2,500 YBP, interrupted by few periods of mesic conditions. Prolonged drought and increased fire risk may have decimated or even wholly destroyed segments of the retreating westernmost disjunct pine islands. The shift towards the warm-moist climate from 2,500 to 1,000 YBP could have caused the Lost Pines to rapidly expand its effective population size (Table 3.5). Another gradual bottleneck may have followed this expansion phase in response to a warm-dry climate from 1,000 YBP to the present (Table 3.5).

The proposed post-Pleistocene scenario for the Lost Pines population expansions and contractions is consistent with pollen profiles and paleoclimate records. The pine pollen profiles in central Texas showed higher quantities toward the late-glacial period 16,000 – 14,000 YBP and lower quantities during the Holocene (Bryant and Holloway, 1985). Similar trends in temperatures and moisture was obtained from other paleo-ecological records (Toomey et al., 1993; Russ et al., 1996).

Table 3.5

A proposed model for the history of the Lost Pines following the paleoclimatic and fossil records in central Texas. Model is showing a series of expansion and contraction in response to climate change.

Time	Paleoclimate	Proposed response	References
Last interglacial period (70,000 – 100,000 YBP)	Prolonged period of interchanging dry warm weather with few cycles of wet cool weather	Populations may have contracted. Contraction was interrupted by slight expansion following climate	Emiliani, 1971
Last Glacial period (11,000 – 70,000 YBP)	Prolonged period of cooler and wetter climate interrupted by few	Populations may have expanded. Expansion was interrupted by slight contraction following climate	Emiliani, 1971
Late Pleistocene and Pre-Holocene (11000 - 21000 YBP)	Cooler and more mesic than present.	Pine population expanded	Graham and Heimsch, 1960; Sorenson et al., 1976; Bryant and Holloway, 1985; Toomey et al., 1993; Bousman, 1998; Musgrove et al., 2001.
Early Holocene 11000-5000 YBP	Cycles of mild wet and dry periods with dry periods of increasing duration	Slight contraction and expansion	Bryant and Holloway, 1985; Toomey et al., 1993; Bousman, 1998; Musgrove et al., 2001.
Middle Holocene 5000-2500 YBP	Prolonged dry period (drier than present conditions) with few short wet periods	Sharp contraction with very brief periods of relief	Bryant and Holloway, 1985; Toomey et al., 1993; Brown, 1998; Goodfriend and Ellis, 2000; Russ et al., 2000
2500-1500 YBP	Warm and wetter than present.	Recovery of the Lost Pines population	Toomey et al., 1993; Brown, 1998; Goodfriend and Ellis, 2000
Late Holocene 1500 YBP-now	Prolonged warm dry conditions with few wet cycles	Milder population contraction than bottleneck caused by the Altithermal.	Toomey et al., 1993; Brown, 1998; Russ et al., 2000

Our results support one or more contractions for the Lost Pines population in central Texas over a geological time scale. Given its rich historical and geological framework, the Lost Pines population illustrates the genetic consequences of population contraction and expansion. Despite one or more bottleneck events, there appears to be no loss in allelic diversity in this resilient colonizer. Results provided no support for the steady-state hypothesis. However, soil characteristics may affect where the Lost Pines population persists today.

Conclusions

At least one genetic bottleneck was detected in the Lost Pines population of *P. taeda* in central Texas. There was no reduction in the allelic diversity of the Lost Pines compared to present-day control from the Western Gulf population. Rare allele loss was detected shifting the allelic distribution of the Lost Pines. Timing of the bottleneck using these tests showed that the bottleneck could have happened during the past 120,000 years. Knowledge of the geological history and paleorecords of central Texas suggests that this bottleneck was most likely a response to a warm dry period of the current interglacial (the past 11,000 years). We proposed that the bottleneck signatures and high allelic diversity of the Lost Pines are the results of contraction and expansion of the Lost Pines parallel changing climatic conditions.

CHAPTER IV

THE ORIGIN OF THE LOST PINES: HISTORICAL PERSPECTIVE AND DNA FINGERPRENTING

Introduction

It is important to verify that the Lost Pines population, a disjunct *Pinus taeda* population at the westernmost edge of the species distribution, is of natural origin. A natural origin is an implicit assumption for any population genetics analysis or conceptual framework. It seems paradoxical to discuss the origin of the Lost Pines although the samples have been analyzed in the previous chapters, especially the bottleneck analysis. However, because this topic was researched within the historical time scale, the order of the time scales was kept intact by moving from a geological time scale to a historical scale. Recent time scales will be discussed in Chapter V.

The Lost Pines origin and the two-refugia hypothesis

During the advancement and retreat of the glaciers during the Ice Age, temperature, marine and vegetation zones were believed to have compressed towards the equator and then shifted back toward the poles in a repeated cycle

(Davis and Shaw, 2001). These climatic oscillations may have shifted conifer species distributions including *P. taeda*. Pollen cores show that boreal and temperate species had their ice-age refugia south of the ice sheets in areas that include central Texas (Jackson et al., 2000).

Fossil pollen for the southern pine species was found in southern Florida (Jackson et al., 2000), indicating the presence of a refugium in that area. The northern extent of the refugium was not determined. Bryant and Holloway (1985) recovered fossil pollen for southern pines from a central Texas bog dating back to 16,000 YBP and this suggested a second refugium in central Texas. The weakness behind this assertion is that the exact species cannot be identified from the pollen samples in the bog. The confinement of *P. taeda* to two refugia is supported by morphological and molecular marker studies (Wells et al., 1991; Schmidtling et al., 1999; Al-Rabab'ah and Williams, 2002).

The Lost Pines origin and the historical records

Historically speaking, the Lost Pines existed in Bastrop County and adjacent counties in central Texas well before the Anglo-American settlers arrived. As early as 1690, an expedition by De Leon and Mansanet went through Fayette County. According to Youngman (1965), De Leon and Mansanet reported "tall trees" in the area where pine trees are currently growing. No further

specifications were mentioned about these “tall trees” (Bolton, 1959). However, there is no other tree in this area that can be called a “tall tree” other than pine (Youngman, 1965). Supporting this conclusion, just one year later, the next expedition in 1691 by Teran de los Rios, a Spanish explorer, went along the Colorado River. They wrote of massive pines growing along the west side of the Colorado River (Bastrop Historical Society, 1955).

Documented descriptions by the Anglo-American settlers in the early 1800’s showed that pure stands of pines were already in Bastrop and the surrounding areas. As a founder of the first Anglo-American settlement in central Texas, Stephen F. Austin first viewed the Bastrop area and the Colorado River in 1821. He described the Colorado River in Bastrop County as “poor gravelly ridges and near the river heavy pine timber” (Fitzwilliam, 1955).

The early naturalists were also aware of the extraordinary presence of those pine trees. Kennedy (1925) described the vegetation of Texas based on data originally published in 1841. He mentioned a stand of “yellow pine” two miles wide extending from north of LaGrange (Fayette County) to the Colorado River just north of Bastrop. Youngman (1965) reported a translation of the original nineteenth-century publication by Roemer, when he visited Texas in 1845 to 1847, indicating the presence of pure pine forests in the Bastrop area.

Pines in central Texas were reported even west and southwest of their present range (Kennedy, 1925; Jones, 1936 in Youngman, 1965), where they no longer grow naturally. In 1807, while Zebulon Pike was being escorted back to the United States by Spanish authorities, he reported occasional pine trees between San Antonio and the Brazos River (Anonymous, 1952); however, it is not clear if those trees were *P. taeda*. Pine forests were also reported by H. S. Morgan west of Austin (Jones, 1936 in Youngman 1965). Based on the 1841 published data, Kennedy (1925) mentioned pine forests in Gonzales and Victoria counties southwest of the present disjunct pine population. In his visit to Texas (1845 to 1947), Roemer reported pine forests in Caldwell and Travis counties (Youngman, 1965).

Records of the early Spanish explorers clearly prove that these pines existed 350 to 400 years ago; however, the possibility of admixture origin has not been ruled out. Seeds brought in by early explorers, Indians, birds as well as natural means are all hypothesized as partial possible causes for the presence of these pines in central Texas (Anonymous, 1952; Bastrop Historical Society, 1955; Wilson, 1962; Youngman, 1965; Moore, 1977) but historical records provide no support.

The Lost Pines origin and DNA fingerprinting

Hypervariable markers, like microsatellites, can pinpoint the origin of unknown samples via DNA fingerprinting (Smouse and Chevillon, 1998; Cornuet et al., 1999; Pritchard et al., 2000; Dawson and Belkhir, 2001; Guinand et al., 2002). DNA fingerprinting tests have been developed for multiple uses, one of which is to assign unknown individuals to the original provenance based on their multilocus genotypes.

The assignment of individuals to their respective populations can be performed using the Assignment Test or the frequency method first developed by Paetkau et al. (1995). This test assigns an individual to the population in which the individual's genotype is most likely to occur based on the allele frequencies of the individual and the population (Cornuet et al., 1999). Another method is a Bayesian method first developed by Rannala and Mountain (1997) to detect immigrants. This method uses the allele frequencies of a population to develop a probability density function from which the marginal probability of an unknown individual will be calculated (Cornuet et al., 1999). A third method, based on measures of genetic distance (Cornuet et al., 1999), estimates a variety of genetic distances between the individual and the populations and then assigns the individual to the closest population (lowest genetic distance).

All previous methods were based on assigning individuals to populations; however, Cornuet et al. (1999) developed what is called an exclusion method where a population will be excluded as the possible origin of an individual. To exclude a population might be more important than assigning the individual to a population, especially in forensics and criminal procedures (Primmer et al., 2000).

A model-based Bayesian clustering method was also developed by Pritchard et al. (2000), where clustering techniques with a Bayesian approach are employed to reveal population structure. This approach can also be used to assign individuals to the closest population based on the highest likelihood or probability (Pritchard et al., 2000). A slight modification to this approach was suggested by Dawson and Belkhir (2001) where the structure of the source populations was treated as unknown.

A non-parametric approach has also been developed to assign individuals to populations using the machine learning approaches such as artificial neural network, decision trees and k-nearest neighbor clustering (Guinand et al., 2002). These methods were compared to the Assignment Test using the likelihood approach of Paetkau et al. (1995) and showed comparable results (Guinand et al., 2002).

A multivariate approach, originally designed for inferring population structures, can be used to infer close relationships between populations. This approach includes principal component analysis or factor analysis (Cavalli-Sforza et al., 1994). This approach was explained in more detail in Chapter II.

To determine the best method for the Lost Pines analysis, we relied on the simulation studies conducted to distinguish between these methods. Although all of these methods can give reliable assignment of individuals to populations, some are more applicable than others. In one study, Cornuet et al. (1999) compares the first three methods described above (the frequency method, the Bayesian method and the genetic distance method). Using simulation studies, the Bayesian method outperforms the frequency method. The genetic distance method performs poorly compared to the previous methods (Cornuet et al., 1999). In practice, the three methods are capable of assigning individuals to populations with a good degree of accuracy (Primmer et al., 2000) but the best choice was the Bayesian method.

In comparing the Bayesian method, frequency and genetic distance methods of Cornuet et al. (1999) to the Bayesian clustering of Pritchard et al. (2000), the latter method outperformed the earlier methods (Manel et al., 2002). However, all these methods are powerful for correctly assigning individuals to their respective populations up to 100% in some cases (Eldridge et al., 2001).

Among the genetic distances method, Cavalli-Sforza and Edwards' (1967) chord distance outperformed other genetic distances like Nei's genetic distances (Nei, 1987) and D_{AS} shared allele distance (Chakraborty and Jin, 1993). The genetic distance developed by Goldstein et al. (1995) performed poorly because it is based on strict mutational assumptions regarding allele lengths (Eldridge et al., 2001).

The study objective is to investigate the origin of the Lost Pines based on the allele frequencies using recent DNA fingerprinting methods to look for a possible admixture origin of the Lost Pines, especially from sources east of the Mississippi River Valley, and to interpret the results in light of the historical record of central Texas. The experimental approach was based on testing population membership of the Lost Pines samples to one of the four main *P. taeda* populations (northeast, southeast, northwest and southwest) or one of the two major regions in the natural range of *P. taeda* (east and west of the Mississippi River Valley) by conducting multivariate approaches, assignment methods, exclusion methods and clustering methods.

Methods and materials

Plant material sources

A total of 130 *P. taeda* samples representing five regions across *P. taeda* range were used in this study. These samples are distributed as follows: 29 samples from the northeastern part of the range (NE), 23 samples from the southeastern part of the range (SE), 25 samples from the northwestern part of the range (NW), 35 samples from the southwestern part of the range (SW) and 18 from the Lost Pines (LP). Samples came from grafted orchards collected originally from natural stands around 1952 to 1970. Needle samples were collected by the Western Gulf Forest Tree Improvement Program.

DNA microsatellites

Lyophilized needle tissue protocol for DNA extraction was based on the approach of Doyle and Doyle (1987). A set of 34 microsatellite markers were randomly sampled from a list of 245 genomic (G), low copy (LC) or undermethylated (UM) microsatellites (Auckland et al., 2002): PtTX2008, PtTX2037, PtTX2080, PtTX2082, PtTX2093, PtTX2094, PtTX2123, PtTX2128, PtTX2142, PtTX2146, PtTX2164, PtTX3011, PtTX3013, PtTX3017, PtTX3019, PtTX3020, PtTX3025, PtTX3030, PtTX3032, PtTX3034, PtTX3026, PtTX3037,

PtTX3118, PtTX4001, PtTX4003, PtTX4009, PtTX4011, PtTX4036, PtTX4046, PtTX4056, PtTX4062, PtTX4093, PtTX4100, PtTX4112. Detailed lab protocols, primer annotation and primer sets are provided in Auckland et al. (2002). PCR products were visualized on silver-stained 6% polyacrylamide denaturing gels. The sizes of the alleles were determined using the pGem molecular ladder (Promega). All marker data were collected by Lisa Auckland, Karen Joyner, Kristin Leach, Jessica Zollinger, Ann Scarpa and Virginia Minihan in the laboratory of Dr. Claire Williams.

Statistical analyses

Basic information on allelic frequencies was obtained from two software packages; GENETIX v. 4.02 (Belkhir et al., 1996) and GENEPOP v. 3.3 (an updated version of GENEPOP v. 1.2, Raymond and Rousset, 1995a).

Genetic differentiation was estimated using principal component analysis (Cavalli-Sforza et al., 1994 pp. 39-42) which visually simplifies the large population x allele frequency matrix using orthogonal principal components or factors. The first factor is the compound variable with the largest variance and the second factor, orthogonal to the first, has the next highest variance. Principal component analysis is based on geometric models which do not require assumptions about whether microsatellites mutate by single base

substitutions (the infinite alleles model or IAM) or by repeat unit changes (the stepwise mutational model or SMM). Principal component analysis (PCA) was based on allele frequencies using PROC FACTOR in SAS 6.12 (SAS Institute, Cary, NC). Standardized scoring coefficients were plotted using Sigmaplot 5.0 (SPSS Inc., 2000).

The Assignment Test of Paetkau et al. (1995) was slightly modified and incorporated in a program called WHICHRUN V4.1 (Banks and Eichert, 2000). First, the four major populations (Northeast, Southeast, Northwest and Southwest) were considered as the baseline *P. taeda* populations and the Lost Pines considered as the unknown population. Second, the northeastern and southeastern populations were considered as one population (eastern) and the northwestern and southwestern populations as the other (western). Based on this broad definition, the Lost Pines samples were assigned to either one of these two populations. The likelihood for each individual in the Lost Pines being in any one of the four populations or the two populations was estimated. In order to evaluate how successfully this test is differentiating between our samples, the same classification approach was conducted to assign each one of the total 112 samples (excluding the Lost Pines) using a jackknife method. The percentage of correct and of erroneous classifications was reported.

The Assignment method based on the likelihood approach, the Bayesian approach and the genetic distance approach are all incorporated in a software package, GENECLASS v. 1.0.02 (Cournuet et al., 1999). An exclusion method based on the three previous approaches is also incorporated in the same software. At first, self-classification of the 112 samples was conducted based on the three approaches. Correct classification into one of the four major regions of the natural range (northeast, southeast, northwest and southwest) can be used to examine the performance of these tests. Since population differentiation is known to be low among populations, the same tests were used to classify the 112 samples into one of the two major regions of the range; east and west of the Mississippi River Valley. The Lost Pines samples were then assigned to their most likely origin or to their closest ancestor population using the Bayesian and frequency approach. All tests were run using 100,000 simulations, and the threshold for exclusion was set to 0.001, specifying 99.9% confidence is required to specify the Lost Pines origin as an eastern provenance.

The Bayesian clustering approach (Pritchard et al., 2000) was part of a software package called STRUCTURE v. 2.0 (Pritchard et al., 2000). First, the data from the 130 samples were used to determine the population structure of these samples. The number of population (k) assumed were varied from 2 to 8

to determine the best value for k. The Lost Pines samples were then assigned to the closest cluster.

Results

The western origin of the Lost Pines population was supported over the alternative, an admixture origin. Within the western populations, the Lost Pines population is closer to the southwestern than to the northwestern population. Despite similarity to western populations, some of the Lost Pines samples showed a unique identity, implying that the population is under some degree of reproductive isolation from the rest of the natural range. Accuracy of the different methods used to classify the Lost Pines samples was relatively high. Accuracy improved when the samples were clustered into two major populations compared to four major populations, implying more substantial genetic differentiation at the Mississippi River Valley. This result was supported by the principal component analysis and the other classification techniques, particularly the Bayesian clustering techniques.

Genetic differentiation east and west of the Mississippi River Valley was supported by the principal components analysis. Using three factors, there was separation between the eastern (SE, NE) versus western (LP, SW, NW) parts of the range (Fig. 4.1). The first three factors in the principal components

analysis accounted for 95.8% of the total variability. Factors 1, 2 and 3 respectively represented 86.4%, 6.2% and 3.2% of the variability in allele frequencies among populations. Eigenvalues corresponding to these factors were 4.32, 0.31 and 0.16 (Fig. 4.1), so the majority of the variability was in the first factor.

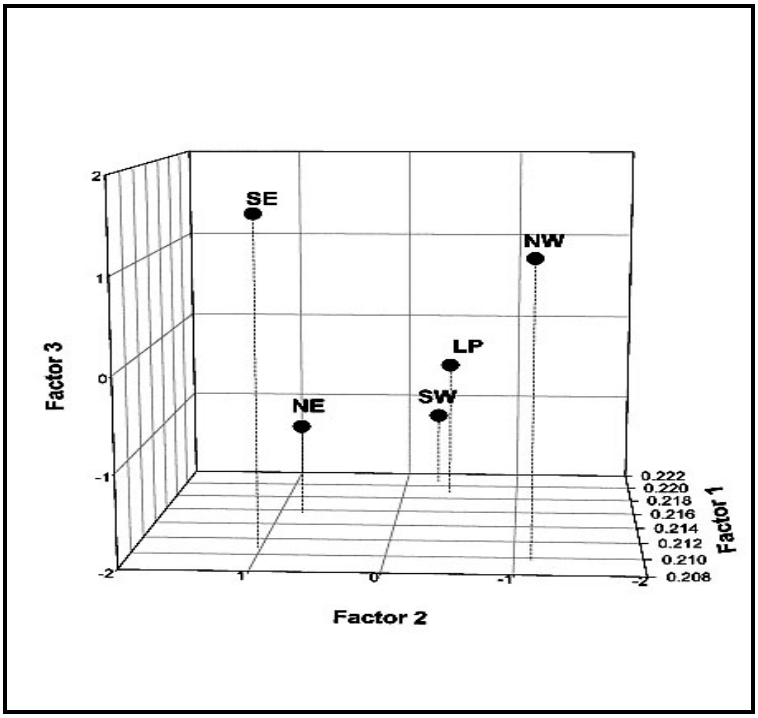

Fig. 4.1. Plot of the first three factors in a principal component analysis based on the allelic frequencies of 18 microsatellite markers scored on the five *Pinus taeda* populations: SE = Southeast, NE=Northeast, NW = Northwest, SW = Southwest and LP = Lost Pines.

Results from the Bayesian clustering method showed that the samples clustered into two major populations; one for the eastern samples, and the other for the western samples (Table 4.1). The northeastern and southeastern samples scored heavily in cluster three (0.233 and 0.708, respectively) and in cluster four (0.544 and 0.42, respectively) (Table 4.1). The northwestern and southwestern populations scored heavily in cluster one (0.276 and 0.725, respectively) and in cluster two (0.639 and 0.203, respectively) (Table 4.1). Given this classification, the Lost Pines population has its highest scores in the western clusters one and two (0.666 and 0.295, respectively), indicating 96% western membership, while it scored only a total of 4% membership in the eastern clusters three and four (Table 4.1). The Lost Pines scored the highest in cluster one (0.666), the same cluster where the southwestern population belong with its highest score of 0.725.

A self-assignment of the samples (excluding the Lost Pines samples) using the Assignment Test (Paetkau et al., 1995; Banks and Eichert, 2000) showed that the classification error rate was reduced from 15.2% to 8.0% only when the samples were classified into one of the two major populations (east and west of the Mississippi River Valley) instead of classifying them into four populations (NE, SE, NW and SW) (Table 4.2).

Table 4.1

Membership proportion of each pre-defined population in each of the inferred clusters. Results show separation between eastern and western populations. The Lost Pines population was closer to the western populations, especially the southwestern population.

	Inferred clusters			
Pre-defined	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Northeast	0.030	0.029	0.233	0.708
Southeast	0.020	0.015	0.544	0.420
Northwest	0.276	0.639	0.057	0.028
Southwest	0.725	0.203	0.039	0.033
Lost Pines	0.666	0.295	0.018	0.021

Table 4.2

Correct classification was improved and error was reduced when samples were classified into one of the two major populations east and west of the Mississippi River Valley (NE = Northeast, SE = Southeast, NW = Northwest and SW = Southwest).

Approach	Classification	Four populations (NE, SE, NW and SW)	Two populations (East and West of the Mississppi River Valley
Assignement Test with jacknife	Correct	84.8%	92.0%
	Erroneous	15.2%	8.0%

Applying the Assignement Test to the Lost Pines samples showed that 100% of the samples (18 out of 18) were classified as a western population with varying degrees of likelihood probability (Table 4.3). Likelihood probabilities ranged form low (~10^{-17}) to high (~10^{-3}), but many folds higher than the eastern

population (~2 to 10^{10}) (Table 4.3). Here too, the Lost Pines showed a closer relationship to the southwestern population than the northwestern population. Out of the 18 samples, 12 showed membership in the southwestern population while six samples only showed membership in the northwestern population (Table 4.3)

Table 4.3

The Lost Pines samples (sample ID) showed 100% coansestry with the western populations. Among the western populations, the Lost Pines showed a greater similarity to the southwestern *P. teada* population.

Sample ID	Classification					
	Four populations Northeast vs. Southeast vs. Northwest vs. Southwest			Two populations Eastern vs. Western		
	Classified	Likelihood	Ratio to the 2nd likely	Pop	Likelihood	Ratio to the 2nd likely
FA1-1	Southwest	0.0027	1.2×10^{4}	Western	4.7×10^{-6}	11
FA1-2	Southwest	9.6×10^{-7}	2.7×10^{4}	Western	3.9×10^{-8}	453
FA2-2	Southwest	4.9×10^{-6}	8.8	Western	9×10^{-8}	2
FA2F24-18	Southwest	6.9×10^{-5}	5.9×10^{8}	Western	2.5×10^{-7}	1×10^{6}
BA1-1	Southwest	0.0022	4×10^{5}	Western	3×10^{-5}	2.2×10^{3}
BA1-2	Southwest	0.0024	134	Western	2×10^{-3}	817
BA3F10-20	Southwest	10×10^{-13}	14	Western	3.7×10^{-13}	6.7
BA3R13-41	Southwest	7.2×10^{-14}	4	Western	1.3×10^{-13}	45
BA5-2	Northwest	2.3×10^{-8}	209	Western	5.1×10^{-8}	4.7×10^{8}
BA3LH-1	Southwest	1.5×10^{-7}	804	Western	3.1×10^{-8}	2.6×10^{3}
BA6-3	Southwest	1.4×10^{-9}	2.4×10^{3}	Western	1.2×10^{-9}	2.2×10^{3}
BA7-1	Southwest	1.2×10^{-6}	3	Western	3.6×10^{-5}	1×10^{6}
BA5-1	Southwest	5.9×10^{-5}	2.7×10^{3}	Western	3×10^{-4}	6.5×10^{7}
1-50	Northwest	2.4×10^{-13}	2.2×10^{10}	Western	5×10^{-17}	1.7×10^{6}
BA2L20-21	Northwest	1.4×10^{-12}	8.9×10^{11}	Western	5.9×10^{-17}	2.4×10^{8}
BA3-1	Northwest	3.3×10^{-14}	3×10^{6}	Western	8.4×10^{-16}	2×10^{5}
D	Northwest	1.7×10^{-11}	5.3×10^{13}	Western	5.9×10^{-17}	5.4×10^{10}
E	Northwest	2.6×10^{-16}	2×10^{6}	Western	6.5×10^{-17}	1.4×10^{14}

The self-assignment approach using the frequency, Bayesian and genetic distance method (Cornuet et al., 1999) also showed similar results. Classification of the samples (excluding the Lost Pines) was improved when two major populations were considered instead of four (Table 4.4). Percent error for classifying samples into four populations was 8.1%, 24% and 12.5% using the Bayesian, allele frequency and chord genetic distance approaches, respectively (Table 4.4). The error rate was reduced for classifying samples into two populations to 5.4%, 9.8% and 8.9% using the Bayesian, frequency and chord genetic distance approaches, respectively (Table 4.4). The Bayesian approach followed by the chord genetic distance approach was more reliable than the allele frequency approach.

Applying these three approaches showed that the Lost Pines samples were assigned to the western population except for a few samples that were not assigned to any population (Table 4.5). The frequency approach showed all samples were assigned to the western population while 16 out of 18 were assigned to the western populations using the Bayesian approach and 13 out of the 18 using the chord distance approach. The remaining two and five samples in the Bayesian and Chord distance approaches could not be assigned to any population with any degree of reliability (< 0.001) (Table 4.5).

Table 4.4

Percentage of correct, erroneous and unclassified samples shows that *P. taeda* can be reliably classified as four major populations, and more reliably as two major populations using nuclear microsatellites.

Approach	Classification	Four major populations	Two major populations
Bayesian	Correct	70.5%	86.6%
	Unclassified	21.4%	8.0%
	Error	8.1%	5.4%
Frequency	Correct	75.0%	87.5%
	Unclassified	1.0%	2.7%
	Error	24.0%	9.8%
Chord Distance	Correct	66.1%	68.8%
	Unclassified	21.4%	22.3%
	Error	12.5%	8.9%

A detailed examination of the Lost Pines alleles frequencies compared to the western population alleles frequencies showed that one allele in the Lost Pines can be considered marginally diagnostic but that there were 12 diagnostic alleles in the western population (Table 4.6).

Table 4.5

The Lost Pines samples classified into one of the two major populations (eastern and western). The Bayesian method assigned 16 out of the 18 Lost Pines samples to the western population. The frequency method assigned all the Lost Pines samples to the western population. The chord distance method assigned 13 out of the 18 Lost Pines samples to the western population. No sample was assigned to the eastern population. Two samples in the Bayeasin method and five samples in the chord distance method were not assigned to any population. Prob. reflects the confidence of the classification.

Sample ID	Classification					
	Bayesian	Prob.	Frequency	Prob.	Chord	Prob.
FA1-1	Western	0.68	Western	0.63	Western	0.18
FA1-2	Western	0.97	Western	0.41	Western	0.01
FA2-2	Western	0.35	Western	0.55	Western	0.08
FA2F24-18	Western	0.99	Western	0.60	Western	0.37
BA1-1	Western	0.96	Western	0.74	Western	0.31
BA1-2	Western	0.99	Western	0.98	Western	0.16
BA3F10-20	Western	0.06	Western	0.08	Western	0.02
BA3R13-41	Western	0.05	Western	0.06	Western	0.01
BA5-2	Western	0.44	Western	0.45	Western	0.16
BA3LH-1	Western	0.94	Western	0.58	Western	0.13
BA6-3	Western	0.28	Western	0.27	Western	0.19
BA7-1	Western	0.99	Western	0.78	Western	0.18
BA5-1	Western	0.97	Western	0.96	Western	0.27
1-50	Western	0.04	Western	0.05	Unclassified	-
BA2L20-21	Western	0.002	Western	0.05	Unclassified	-
BA3-1	Western	0.003	Western	0.05	Unclassified	-
D	Unclassified	-	Western	0.05	Unclassified	-
E	Unclassified	-	Western	0.05	Unclassified	-

Table 4.6

Diagnostic alleles ($q \geq 0.10$) for the Lost Pines and the western population of *Pinus taeda*. Bold numbers show the only unique allele for the Lost Pines population. Total number of private alleles ($q > 0.05$) is also reported.

Marker	Allele Number (bp)	Allele Frequency	
		Lost Pines (allele copies)	Western Gulf (allele copies)
PtTX2008	316	0.00	0.18 (19)
PtTX2082	227	0.00	0.17 (17)
PtTX2164	269	0.00	0.12 (13)
	248	0.00	0.10 (10)
PtTX3032	**308**	**0.10 (3)**	**0.00 (0)**
PtTX3034	194	0.00	0.10 (11)
PtTX3037	167	0.00	0.10 (10)
	165	0.00	0.12 (12)
PtTX4001	223	0.00	0.16 (18)
	220	0.00	0.13 (15)
PtTX4009	288	0.00	0.11 (12)
PtTX4046	242	0.00	0.21 (23)
PtTX4056	457	0.00	0.12 (13)
Total diagnostic		1	12
Total private		3	28

Discussion

Most of the Lost Pines samples clearly shared ancestors with *P. taeda* populations west of the Mississippi River Valley. The hypothesis for an admixture from eastern provenance was not supported by either the historical

record nor by DNA analysis. However, some of the Lost Pines samples did not show a close relationship to any other population, indicating that the Lost Pines population is evolving into a distinct population possibly the result of reproductive isolation or inter-specific hybridization. This relates to the earlier conclusion that prevailing west to east wind direction may contributed to reproductive isolation based on diagnostic allele distribution. Genetic differentiation at the Mississippi River Valley was also supported using clustering techniques and assignment methods.

The separation between eastern and western populations was shown earlier using morphological and molecular marker data (Wells and Wakeley 1966; Florence and Rink, 1979; Wells et al., 1991; Schmidtling et al., 1999; Al-Rabab'ah and Williams, 2002). Principal component analysis supported this modest genetic differentiation but the differentiation was also apparent for the Bayesian clustering technique (Pritchard et al., 2000). Despite long distance gene flow, populations east and west of the Mississippi River valley possessed a different genetic structure which was revealed clearly using the Bayesian clustering approach. The Assignement Test (Paetkau et al., 1995; Banks and Eichert, 2000) and the assignment and exclusion methods (Cornuet et al., 1999) showed lower error rates assigning the 112 samples from the four main populations (northeast, southeast, northwest and southwest) into two populations (eastern and western) than assigning the same samples into their

own respective population, re-emphasizing the separation between eatsern and western populations.

Most of the Lost Pines samples could be assigned to western sources using multiple methods. Several Lost Pines samples were assigned to western populations with a low probability. These results suggest two explanations.

First, the sample sizes for the source population need to be larger. The exclusion method indicated that the source population was either not sampled adequately when a sample cannot be assigned to any of the source populations or that there is a missing baseline population. Larger sample sizes in the basline population are needed.

Second, the lack of classification could indicate new mutations among the Lost Pines that have not yet been moved into the larger *P. taeda* populations by prevailing winds. A related possibility is that some of the Lost Pines samples are from inter-specific hybridization or introgression.

The fact that the Lost Pines samples showed clustering with mainly southwestern populations followed by northwestern populations implies that these three populations have originated from a common ancestral population. These results along with previous genetic results support the presence of a

second *P. taeda* refugium population in Texas during the Last Glacial Maximum (LGM). This refugium may have radiated or expanded to become current western populations when glaciers retreated to northern lattitudes. These results complement pollen data that showed fossil pine pollen from subsection *Australes* in central Texas 16,000 years ago (Bryant and Holloway, 1985).

Assigning Lost Pines samples into their respective populations showed the relative power of these tests for assigning each individual to the closest population. The Bayesian method had the highest probability of correctly assigning individuals to the correct source followed by the allele frequency method. The least powerful was the chord genetic distance method (Cavalli-Sforza and Edwards, 1967) for estimating genetic distnaces. These results support simulation studies comparing these approaches in assigning individuals to their likely sources (Cornuet et al., 1999; Eldridge et al., 2001; Manel et al., 2002).

Conclusions

In summary, the Lost Pines samples shared coancestry with the western *P. taeda* populations with no admixture from eaastern populations. The western *P. teada* populations showed significant differentiation from eastern

populations reemphasizing previous conclusions of the existence of a second *P. taeda* refugial population in Texas. DNA fingerprinting are powerful tools assigning individuals to their likely source and revealing population structure.

CHAPTER V

RECONSTRUCTING POPULATION DYNAMICS OF THE LOST PINES USING REMOTE SENSING AND GIS

Overview

Recent population dynamics of the Lost Pines were investigated using aerial photographs, LandsatTM classified image and soil thematic layers. Pine forest distribution in Bastrop State Park and Buescher State Park at two points in time (1949 versus 1995) reflected a response to the absence of logging. Pine forest distribution at the broader county level reflected a complex response to logging and natural conditions. With an absence of logging within the state parks, pine cover increased by 50% from 1949 to 1995. When pine forest cover increased, patches merged and decreased in number. New patches appeared beside the formation of the larger merged patches. For pine patches present in 1949, 36% of them disappeared by 1995. About 53% of the 1995 patches were not present in 1949. The dynamics and distribution of the pine forest shifted in the absence of logging. The distribution of the present-day Lost Pines population in central Texas was mildly limited by specific soil characteristics. This constraint on pine distribution was tested by measuring occurrence of pine stands across all soil types in the area. At the county level, pine forests grew on sandy light

top soils, clayey heavy sub-soils and highly permeable soils. Pine forests grew on other soil types as well. *Pinus taeda* forests were most abundant on soils that ameliorate the drought-prone precipitation regime in this area.

Introduction

Pinus taeda is the predominant coniferous species in the southern quadrant of the United States, providing a major source of wood and fiber (Shultz, 1999). The Lost Pines population is a disjunct *P. taeda* population at the westernmost edge of *Pinus taeda* species range located in central Texas, near Bastrop. Understanding the dynamics of this species in response to environmental change over long geological and historical time scales has been the focus of the previous chapters. Understanding pine population dynamics at the recent time scale due to logging and to soil characteristics provides amore complete picture of ecological shifts at the landscape level.

An indirect estimate of the impact of humans through logging can be measured by the population growth in and around Bastrop. The population of Bastrop fluctuated depending on the historic circumstances. The population of Bastrop County grew from 2,180 in the 1850 to approximately 27,000 by 1900. During the same period, the number of farms increased from approximately 600 to 3,500 farms in 1900 (Gannett, 1902 pp. 12). Due to the Great Depression, the

population dropped to 21,610 by 1940 with only 2473 farms (Bastrop Historical Society, 1980; Robinson, 2001 pp. 23). Since then, the population of the county has risen. Between 1940 and 1947, the county was rejuvenated by the establishment of Camp Swift, a military camp to house 30,000 to 40,000 soldiers during World War II. The camp was built on a land just north of Bastrop State Park (Robinson, 2001 pp. 23 -26).

Logging regimes during the last 200 years in the history of Bastrop County may have had a significant impact on the Lost Pines. During the 1800's and early 1900's, the Lost Pines were logged frequently and sometimes excessively (Easton, 1947; Anonymous; 1952; Bastrop Historical Society, 1955; Youngman, 1965).

Despite heavy logging in the Lost Pines, estimates of the Lost Pines population size were almost the same between the mid 19th century and the mid 20th century (Easton, 1947; Anonymous, 1952). The Lost Pines forest was estimated to occupy about 36,400 hectares in 1880 (Easton, 1947 pp. 93) and about 34,400 hectares in 1952 (Anonymous, 1952); However, the pine lumber production may have been cyclical due to alternating logging and regeneration periods as inferred from various records and sources for Bastrop County and Texas (Anonymous, 1952; Williams, 1989 pp. 274-275, 436-437, 462, 468-477; Marks, 2003). Up until the mid 20th century, logging companies were able to

produce a sustainable amount of lumber from the Lost Pines area each year (Easton, 1947; Bastrop Historical Society, 1955 pp. 6). For example, a Bastrop saw mill produced 30 million board feet (MBF) of lumber from 1928 to 1932 (Marks, 2003).

Bastrop State Park was built along with Buescher State Parks by Civilian Conservation Corps (CCC) between 1933 and 1937 (Barkley, 1970 pp. 90; Medlar, 1995). This area occupies 1,418 hectares. Bastrop State Park was part of the original 1832 land grant to Stephen F. Austin's first colony (Barkley, 1970 pp. 90). Logging ceased with the formation of the state parks, providing an opportunity to study the impact of the absence of logging on the Lost Pines. This novel opportunity was possible because of aerial photographs for the state parks dating back as early as 1949.

Although logging continued outside the park, planting was also underway. The estimated pine-oak area in Bastrop County declined 7% between 1981 and 1999 (Engle, 2002 pp. 20) in response to logging and other factors. Since there was more than one factor affecting the dynamics of the Lost Pines in Bastrop County outside the state parks, the use of the pine distribution in the whole county to study the impact of logging alone was hindered due to confounding impact from other factors.

Pinus taeda trees can live up to 300 to 400 years with a generation interval of 15 to 30 years. This upper limit of the generation interval is extracted from the regeneration habit of *P. taeda* especially in central Texas. *Pinus taeda* seedlings can thrive in open areas created mainly by fire. In fact, the fire regime of this area showed that a fire cycle of less than 10 years and more than 35 years is likely to favor other vegetation types over *P. taeda* (Quarterman and Keever, 1962; Pyne et al., 1996; Wade et al., 2000). A large fire in May of 1984 consumed thousands of acres in the Lost Pines area including the state parks (Terry, 1991). This should be taken in consideration for interpreting pine forest dynamics. Fire is also hypothesized to have enhanced pine forests expansion.

The distribution of the Lost Pines population in central Texas has been attributed to the distribution of suitable soil characteristics (McBryde, 1933; Youngman, 1965; Moore, 1977). The soils of Bastrop State Park are mainly classified as the Axtell-Tabor soil association with roughly about 70% Axtell and 20% Tabor. The Axtell soils have a gravelly sandy loam surface layer approximately 35 cm thick with a red mottled clay subsoil (Baker, 1979). Tabor soils have a gravelly fine sandy loam surface layer approximately 45 cm thick with an underlying subsoil of brownish yellow, mottled clay (Baker, 1979).

Records of ground water levels in Bastrop County varied from as low as one meter (m) below soil surface to about 66 m in various places in the county during different times of the year (Swartz, 1956; Follett, 1970). Water level in and around the Lost Pines area varies from shallow to deep (Swartz, 1956; Follett, 1970).

The availability of current aerial photographs and soil maps for the Lost Pines area provides an opportunity to study the dynamics of the Lost Pines on a landscape basis and to investigate the relationship between the pine forest distribution and soil/site characteristics. Historical and recent aerial photographs are valuable resources for tracking and documenting vegetation or species dynamics and changes (Hudak and Wessman, 1998; Kadmon and Harari-Kremer, 1999; Taylor et al., 2000; Petit and Lambin, 2001).

The objectives of this study were as follows:

1) To investigate the dynamics of the Lost Pines in Bastrop and Buescher State Parks between 1949 and 1995 using historical and recent aerial photographs. To do this, spatial patterns for the parks during this time period were investigated.

2) To investigate the correspondence between the spatial distribution of pine patches and certain soil types.

Methods and materials

Source of aerial photographs, thematic images, soil and weather data

Black and white historical aerial photographs for Bastrop State Park and Buescher State Park dating back to 1949 are archived at the Texas Digital Aerial Photo Archive (TxDAPA), a project under Texas Natural Resources Information System (TNRIS). These photographs were taken in the winter of 1949, which maximize a season differences between evergreen conifers and deciduous forest species. The nominal scale of these aerial photographs is 1 to 15,840, and they were scanned into digital format at 500 dots per inch (dpi) for a final resolution of 0.8 m (Hudak and Wessman, 1998). The resolution is calculated as

$$\text{Resolution (m)} = 15840 / 500\text{dpi} \times 39.37\ \text{in/m} = 0.8\ \text{m} \qquad [5.1]$$

Orthorectified and georeferenced color infra-red photographs (DOQQ) with three bands (green, red and infra-red) for the two state parks were obtained for 1995 from TNRIS. Aerial photographs from 1949 and 1995 share a similar

spatial resolution ideal for detecting vegetation change for a relatively small area.

A classified LandsatTM image (Earth Observation Satellite Company, 1994) for east and central Texas dating back to 1992 was provided by the Spatial Science Laboratory (SSL) at Texas A&M University (unpublished data). A subset image for Bastrop County was taken from that image with a spatial resolution of 30 m. This thematic image was classified into different categories, including pines as one major category but no groundtruthing was conducted for this image. Groundtruthing refers to the process of collecting control points on the ground and comparing them to the classified image to estimate classification accuracy. Pines in east Texas include several pine species, but *P. taeda* is the prevalent pine species in Bastrop County.

Soil information was obtained from the Soil Survey Geographic Data Base (SSURGO) provided by the United States Department of Agriculture/Natural Resources Conservation Service (USDA, 1995). The spatial resolution of these data is approximately 30 m. Weather information was extracted from the monthly climate data provided by the National Climatic Data Center (NCDC) for Bastrop County (NCDC, 1989).

The 1995 DOQQ photographs cover the two state parks area only. For a county-wide coverage, a 1992 classified landsatTM image was used. The resolution of the 1992 image is similar to the resolution of the soil data (30 m).

The aerial photographs and soil data were projected to the Universal Transverse Mercator (UTM) projection, zone 14 or 15 with reference to the Geodetic Reference System (GRS) 1980, the North American Datum (NAD) 1983 using the units of meters.

Georeferencing the historical aerial photographs

Aerial photographs for Bastrop State Park and Buescher State Park taken in 1949 were georeferenced using the georeferenced 1995 DOQQs. The procedure suggested by the TxDAPA for georeferencing historical photographs (http://www.tnris.state.tx.us/ResearchCenter/RemoteSensing/txdapa.htm) was followed with some modifications.

Georeferencing was first done by locating ground control points (GCPs). GCPs are based on common features preserved across the time period from 1949 to 1995 such as roads, intersections, field boundaries and urban structures. Many landscape features had changed between 1949 and 1995. Collecting GCPs for

georeferencing was challenging because state parks do not have consistent roads, intersections, buildings or other entities that could serve as GCPs.

This problem was solved by using GCPs from two nearby towns, Bastrop and Smithville. The town of Bastrop lies at the western edge of Bastrop State Park, and the town of Smithville lies at the southern edge of Buescher State Park (Fig. 5.1). Most of the roads and intersections within these two towns have been preserved over this time period aided collection of at least 25 GCPs (see Fig. 5.2 and 5.3). These GCPs were used to georeference all the photographs that shared any part of the two towns with an error of less than 0.2 m using a second order polynomial (ERDAS, 1999). The overlap between the town photographs and the adjacent park photographs to the east of Bastrop and to the north of Smithville was utilized to georeference all images for these two state parks. At least 15 well-distributed GCPs were used to georeference each photo with a maximum total spatial error of two meters (ERDAS, 1999).

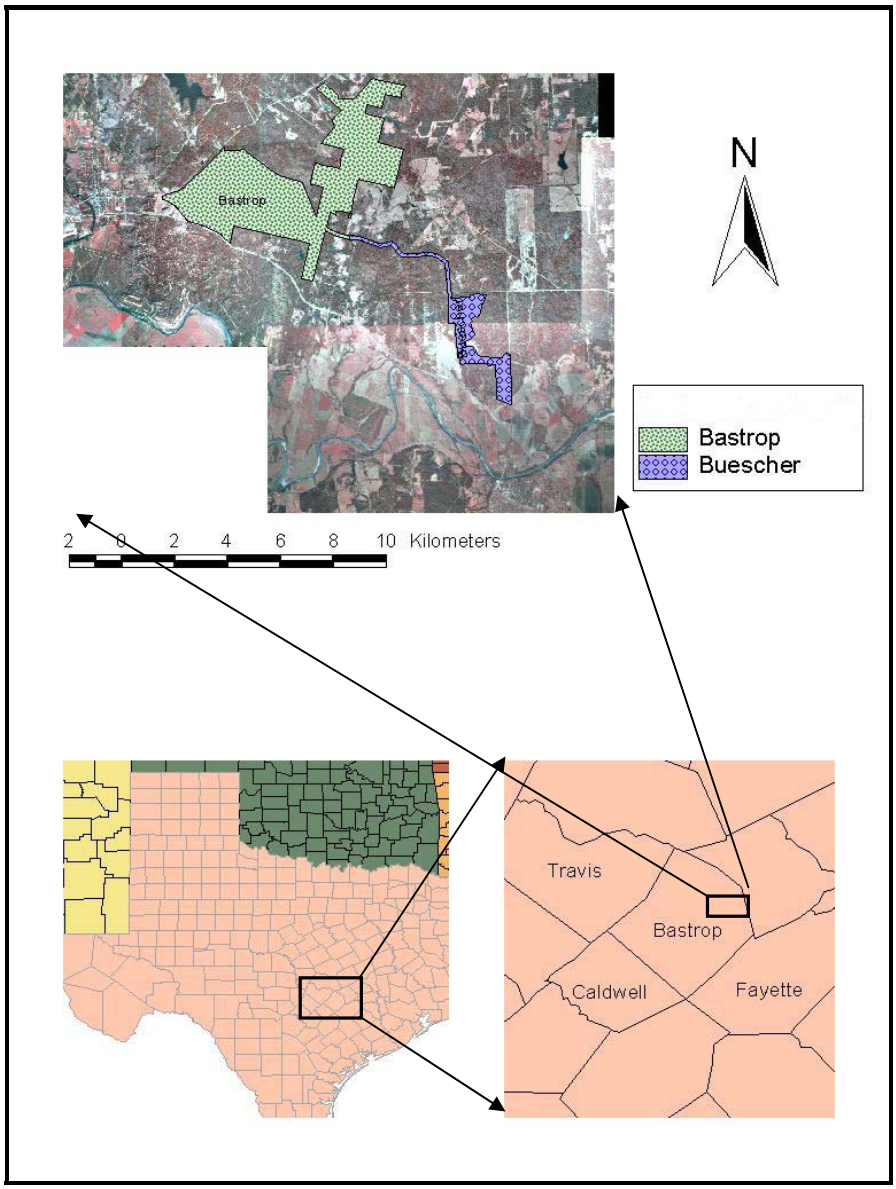

Fig. 5.1. Map showing the position of Bastrop State Park and Buescher State Park in relation to the towns of Bastrop and Smithville and the Colorado River in Bastrop County, Texas.

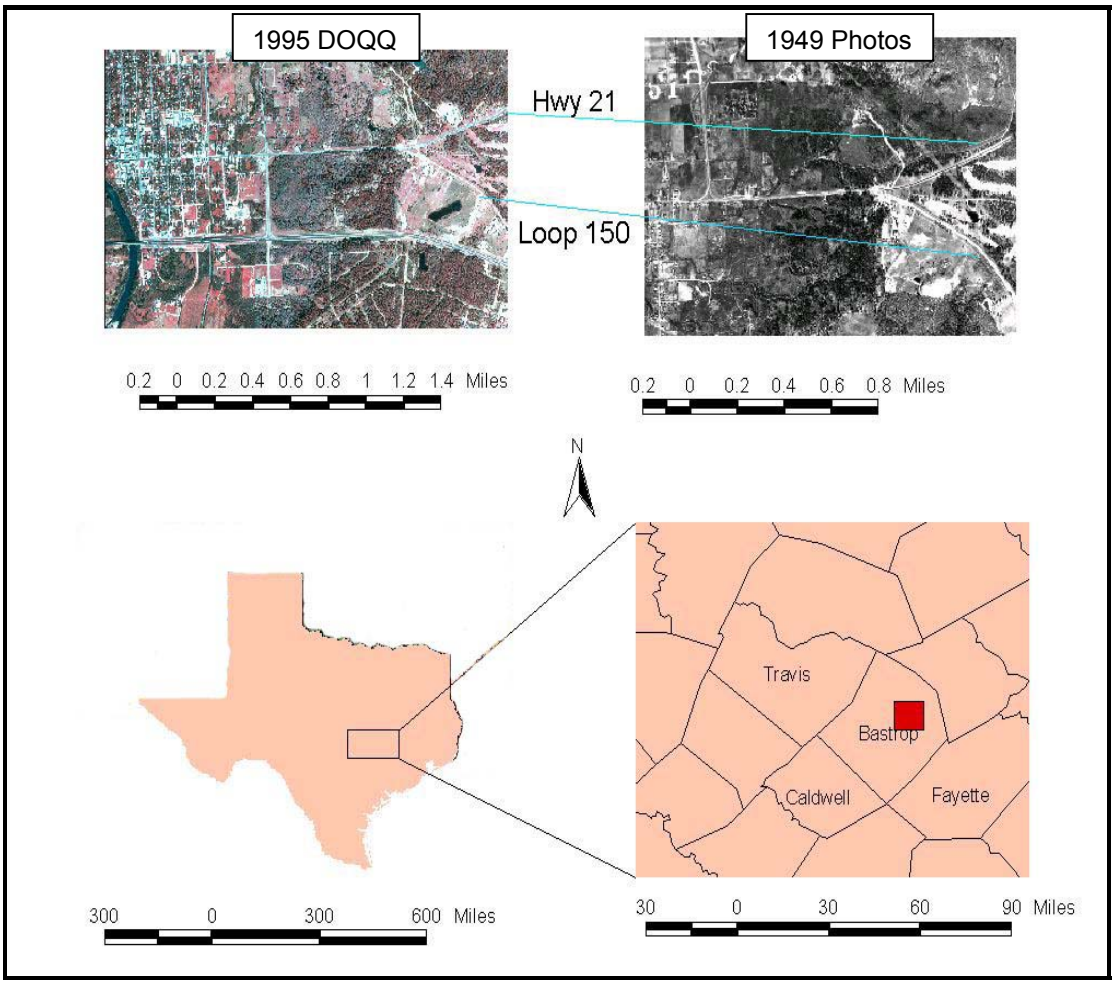

Fig. 5.2. Map showing the town of Bastrop, with the major streets and intersections preserved as GCPs for georeferencing the 1949 aerial photos to the 1995 Digital Orthorectified Quad Quads (DOQQs).

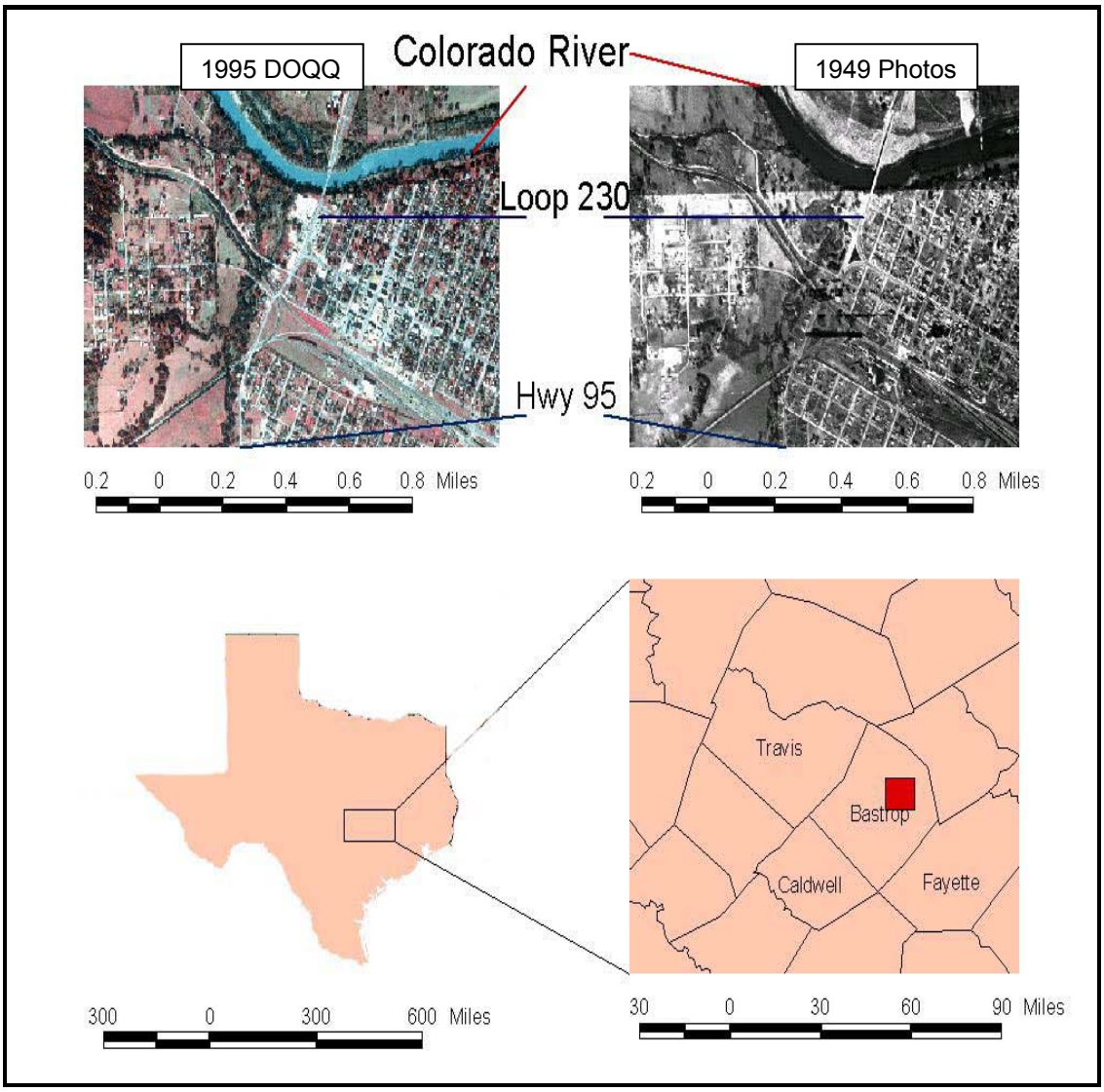

Fig. 5.3. Map showing the town of Smithville, with the major streets and intersections preserved as GCPs for georeferencing the 1949 aerial photos to the 1995 Digital Orthorectified Quad Quads (DOQQs).

Image processing and classification

Both the 1949 and the 1995 aerial photographs were re-sampled from the original 0.8 m resolution to 2 m to reduce noise associated with finer resolution. Images were then individually classified to 100 initial classes using the unsupervised classification method built in ERDAS 8.5 (ERDAS, 1999) using the Isodata algorithm with a convergence threshold of 0.95 and maximum iteration of 15. The maximum iteration was not reached because the minimum convergence threshold was satisfied before reaching the maximum iterations. These 100 classes were re-coded into two main classes; pine and everything else (other forest types and bare soil). These 1949 and 1995 classified images were then grouped into mosaics to generate two images; one for the 1949 pine distribution and one for the 1995. These two images were re-sampled again to 30 m resolution to match the resolution of the soil data.

Both the 1949 and 1995 images were subset to the boundaries of the Bastrop State Park and Buescher State Park. The subset images were then converted to shape files and to grid coverage using Arcview 3.2 (Redlands, CA). Several landscape measures were then calculated using FRAGSTAT Spatial Pattern Analysis program (McGarigal and Marks, 1994; Elkie et al., 1999).

Accuracy assessment

Accuracy assessment for the validity of the classification for the 1949 and 1995 photos was conducted using photo interpretation. This method consists of randomly choosing 250 points on the photos and visually determining their vegetation class. The vegetation class for these 250 points was compared to the vegetation class in the classified image, and the error percentage was calculated. Using our classification scheme, error rate was 12% (88% accurate classification). Part of this error is due to mistakenly classifying other vegetation types into pines (Commission error). Commission error was 2% only. The other type of classification error is the omission error (pines are being mistakenly classified into other vegetation types). Omission error was 10% error. The low rate of commission error relative to the rate of omission error is conservatively underestimates pine forest area for 1949 and for 1995 but this error rate should have a minimal effect on the analysis.

Classified image analysis

Various types of comparative spatial pattern analyses were conducted between the 1949 and the 1995 classified images to test the impact of human logging on the pine distribution. First, the pine area (class area or CA) for the 1949 and 1995 was calculated along with the total area of the two state parks (TA). The

percentage of pine area relative to the total area (CA/TA) in the two state parks represents the composition of the landscape (or the two state parks) with no reference to the configuration of the pines within the landscape (McGarigal and Marks, 1994; Elkie et al., 1999).

To examine the spatial dynamics of the pine patches between 1949 and 1995, the 1949 spatial location of the pine patches was compared to 1995 spatial locations. Percentages of persistent and non-persistent pine patches between 1949 and 1995 were calculated. Percentage of new pine patches observed in 1995 was also calculated.

Landscape metrics of interest to this study have been calculated for the 1949 and 1995 images. The number of pine patches (NumP) and the mean patch size (MPS) were measured. Both NumP and MPS together can provide information about the parks' configuration disregarding the spatial position. For example, NumP and MPS can be compared for the same area to see whether the number and size of pine patches increased, decreased or remained the same during a certain period of time. The NumP and MPS values may stay the same with no change; however, the spatial location of the pine patches could have been changed dramatically without changing the values of NumP and MPS. These two measures can provide a measure of the total pine area (TA = NumP x MPS). If the values of NumP and MPS change, this implies that pine

patches are changing. The largest patch index (LPI) is a measure of the area of the largest pine stand which can serve as an index measuring stand dynamics. Decreased LPI value may indicate fragmentation while increased LPI value may indicate growth or aggregation.

Edge metrics are other measures of landscape configuration without any reference to the spatial position. They measure the amount of edge around the patch, a boundary area relevant to wildlife management (McGarigal and Marks, 1994; Elkie et al., 1999). Some wildlife species depend on the edge environment of a patchy landscape, and so the amount of edge rather than the amount of a specific class or classes is an important variable (Logan et al., 1985). Total edge (TE) for pine patches was calculated and then used to calculate the mean patch edge (MPE) as the ratio of the total edge (TE) to the number of patches (NumP). The MPE value indicates how many meters of edge a pine patch has on average.

Shape metrics are relevant in ecological research but difficult to quantify concisely (McGarigal and Marks, 1994). Shape metrics usually contain information about the perimeter-area relations. Shape metrics give an indication of whether the shape is regular or complex. The area-weighted mean shape index (AWMSI) is an index where the ratio of a pine patch perimeter to its area is compared to the same ratio in a simple shape (e.g. circle). The index

will be equal to one if the shapes are perfectly regular and will be greater than one if they are complex. This index is weighted by the area of the patch to give more weight to large patches. The area-weighted mean patch fractal dimension (AWMPFD) is in principle the same as the AWMSI with a different approach. Fractal dimension analysis is useful for studying hierarchical systems when applied across scales (Milne, 1988). Reporting this index in one scale indicates the complexity of the pine patches.

Core metrics are another useful landscape metric which measure the area of a pine patch after buffering a certain distance from the edge. Core metrics indicate the effective patch area after buffering rather than the exact patch area. Core metrics reflect landscape composition and configuration. For example, a fragmented landscape with small patches of a certain species might have the same area as another landscape with larger patches. For the former landscape, core metrics such as the core area will be small after buffering a certain amount of the circumference of the patch. For the later landscape, core area metric will be larger after buffering the same value from the circumference of the patches due to the larger patches in the second landscape. In contrast to the edge metrics which are useful for edge related species, core metrics are important for interior species which prefer habitats away from the edges (Hansen and di Castri eds, 1992).

Total core area (TCA), number of core area (NCA), mean core area (MCA) and core area density (CAD) were calculated. TCA was calculated as the total core area for pine patches after buffering a border of 20 m. NCA is the number of pine patches containing a core area after buffering. If a patch is lessr than 20 m wide, it will not have a core area. Some of the patches are less than 20m wide, so NCA is expected to be less than the absolute number of patches (NumP). MCA is the mean area for all the pine core areas in the state park calculated as the TCA divided by the NCA. CAD is a measure of how many pine core areas (pine patches more than 20m wide) one can find in a certain area (100 ha).

Soil and weather data preparation and analysis

Soil survey data (SSURGO) provided by the United States Department of Agriculture/Natural Resources Conservation Service (USDA, 1995) for Bastrop County were imported into Arcview 3.2. For comparison purposes, SSURGO data for soils of the pine patches and of the other classes were compared for the state parks using the 1949 and 1995 images and for Bastrop County using the 1992 image. Different soil attributes were considered for comparing the surface soils and the sub-soils (Table 5.1). These attributes represent important soil factors thought to affect the distribution of pines (Wahlenberg, 1960; Farmer, 1988; Shultz, 1997).

The 1995 pine-soil distribution represents the relationship of pine to soil characteristics within the park. The 1992 whole county pine-soil distribution represents the relationship of pine to soil conditions in addition to logging and other human influences. Comparison between these two distributions (park and county) can be made only if the park soils represent the county soils.

Table 5.1

Description of the soil properties used in the study.

Element	Surface or layer	Description
Texture	Surface	Code for the USDA texture classification for the surface layer.
Drainage	Surface	Code identifying the natural drainage condition of the soil and refers to the frequency and duration of periods when the soil is free of saturation.
Slope	Surface	Value representing the average slope.
Erosion	Surface	Code for the condition of the soil erosion (Not eroded; eroded; severely eroded).
Clay	Layer	Value representing the average clay content as a percentage from the total weight (gram clay/gram soil x100).
CEC	Layer	Value representing the average cation exchange capacity (meq/100g).
Permeability	Layer	Value representing the average rate of permeability (25.4mm/hour).
BD	Layer	Value representing the average moist bulk density (g/cm^3).
PH	Layer	Value representing the average soil reaction (pH).
OM	Layer	Value representing the average organic matter content (%/wt)

To test the significance of the difference between characteristics of soils inside the park and county soils, random points from the soil thematic image were collected inside and outside the parks. Fifty points were randomly sampled for soil variables with less than five classes. For variables with greater than five classes, 100 random points were sampled to ensure that each class of any soil variable will have at least three to five samples. Random point samples from these images and thematic layers were made possible using ERDAS 8.5 (ERDAS, 1999).

Frequency of different classes of a soil variable was calculated. Frequencies obtained from the park samples represent the observed frequencies. Frequencies obtained from the county samples are considered expected frequencies. If soils of the two parks are representative of the whole county, then a test of goodness of fit between the park samples (observed) and the county samples (expected) will show a good fit with no significant difference. The G-test, adjusted with Williams' correction for a conservative test, for the goodness of fit was used (Sokal and Rohlf, 1995 pp. 685-708). The G-test approximates the chi-square distribution. This test determines which soil attribute in the two parks is a random representative of the soils in the whole county.

To test the absence of logging on the pine forest distribution across soil types, the 1949 pine forest distribution was assumed to be affected by prior logging history while the 1995 pine forest distribution was assumed to be a response after logging cessation. Based on these assumptions, the absence of logging on the distribution of pines could be tested relative to the soil attributes. Random points (50 or 100) were selected from the pine coverage in 1949 and from the pine coverage in 1995. The difference between the soil variables was tested using the G-test.

To test the relationship between pine forest distribution across soil types, the same adjusted G-test was used. Comparison was made between soil characteristics with pine patches compared to the general soil characteristics at the county level and at the park level separately. Randomly sampled points (50 or 100) were used to represent the soils in the county. Other randomly sampled points were used to represent the soils with pine patches.

Frequencies of different soil classes from pine soils (observed) were tested against frequencies of the same soil classes from the general county soils (expected). The same test was performed for state park soils. The 1992 classified image was used for the Bastrop County test. The 1995 classified aerial photographs (DOQQs) were used for the state parks.

Weather data were imported into Arcview 3.2 as data points from six different weather stations distributed in Bastrop County. Data were thoroughly checked for typos, repetitions or missing values. Any record for any weather data element (i.e. a variable like temperature or precipitation) that had four or more months missing in one year was excluded. Any weather element that had not been measured for at least 40 years between 1950 and 1995 was also excluded.

Data elements were then summed and averaged for each station over the period of 62 years from 1940 to 2001. Various weather elements have been reported to affect pine distribution. Most of these elements are related to precipitation and temperature (Hocker, 1956; Wahlenberg, 1960; Walker and Oswald, 2000). Of these elements, 11 have met the criteria and showed acceptable representation throughout years and stations (Table 5.2).

Table 5.2
Description of the weather elements used in the study. Weather elements are related to precipitation and temperature.

Weather element	Description
DP10	Number of days with greater than or equal to 25.4 mm precipitation
DSNW	Number of days with snow depth greater than or equal to 2.54 centimeter (cm)
DT00	Number of days with minimum temperature less than or equal to –17.8 °C
DT32	Number of days with minimum temperature less than or equal to 0 °C
DT90	Number of days with maximum temperature greater than or equal to 32.2 ° C
DX32	Number of days with maximum temperature less than or equal to 0 °C
MMNT	Monthly mean minimum temperature (°C)
MMXT	Monthly mean maximum temperature (°C)
MNTM	Monthly mean temperature (°C)
TSNW	Total monthly snowfall (mm)
TPCP	Total precipitation (mm)

Results

The Lost Pines population in the Bastrop State Park and Buescher State Park showed a significant increase in the total pine forest area in the period between 1949 and 1995. This increase in total pine forest area was associated with 1) a decrease in the number of pine patches and 2) a significant increase in the mean area for each patch. Area, edge, shape and core metrics for pine patches showed substantial change with the absence of logging. These metrics indicated that *P. taeda* patch area, edge, complexity and core area all increased between 1949 and 1995. *Pinus taeda* in the two state parks showed

a significant change. For the pine patches in 1949, 36% of them had disappeared by 1995, and 53% of the 1995 patches had formed after 1949 in new areas. *Pinus taeda* forest are over-represented on certain soil types such as the sandy light top soils underlain by clayey heavy subsoils but the species thrives outside these specific soil types as well.

Pine forest dynamics in Bastrop and Buescher State Parks

The combined study area for the two state parks is approximately 2,610 hectares (ha) excluding a small area from Bastrop State Park due to lack of aerial photograph coverage. *Pinus taeda* patches in 1949 made up 25.5% of the total area of the two state parks. In 1995, *P. taeda* patches made up 37.2% of the total area (Table 5.3). This increase in the total pine area was accompanied by a decrease in the number of pine patches from 1333 to 808, but the mean area for each patch significantly increased from 0.5 ha to 1.2 ha. This increase in the mean patch area accounted for the total increase in the pine area. *Pinus taeda* patches in general increased in their size from 1949 to 1995 (Table 5.3). The percent of *P. taeda* area that existed in 1949 and still existed in 1995 was 64% of the 1949 pine area, while the rest of the 1949 pine area (36%) had disappeared by 1995. The percent of new *P. taeda* patches formed after 1949 and still existed in 1995 comprised 53% of the 1995 pine area. Note that the pine area in 1949 is different from 1995 so percentages

relative to 1949 are different from those relative to 1995. An example of how small *P. taeda* patches grew up and formed larger patches is presented in Fig. (5.4).

Table 5.3
Spatial statistics for the 1949 and 1995 *P. taeda* patches in Bastrop and Buescher State Park showing a significant change during the last 45 years.

Spatial Statistics	**1949**	**1995**
Class Area (CA, ha)	665	970
Total Area (TA, ha)	2610	2610
% Pine	25.5%	37.2%
Number of Patches (NumP)	1333	808
Mean Patch Size (MPS, ha)	0.5	1.2
Total Edge (TE, km)	566	722
Mean Patch Edge (MPE, m/patch)	425	894
Area Weighted Mean Shape Index (AWMSI)	6	14
Area Weighted Mean Patch Fractal Dimension (AWMPFD)	1.23	1.32
Mean Nearest Neighbor (MNN, m)	35	33
Total Core Area (TCA, ha)	28	54
Number of Core Area (NCA)	146	304
Mean Core Area Index (MCAI)	0.21	0.44
Core Area Density (CAD, number/100ha)	6	12
Largest Patch Index (LPI, percent)	3.6	8.8

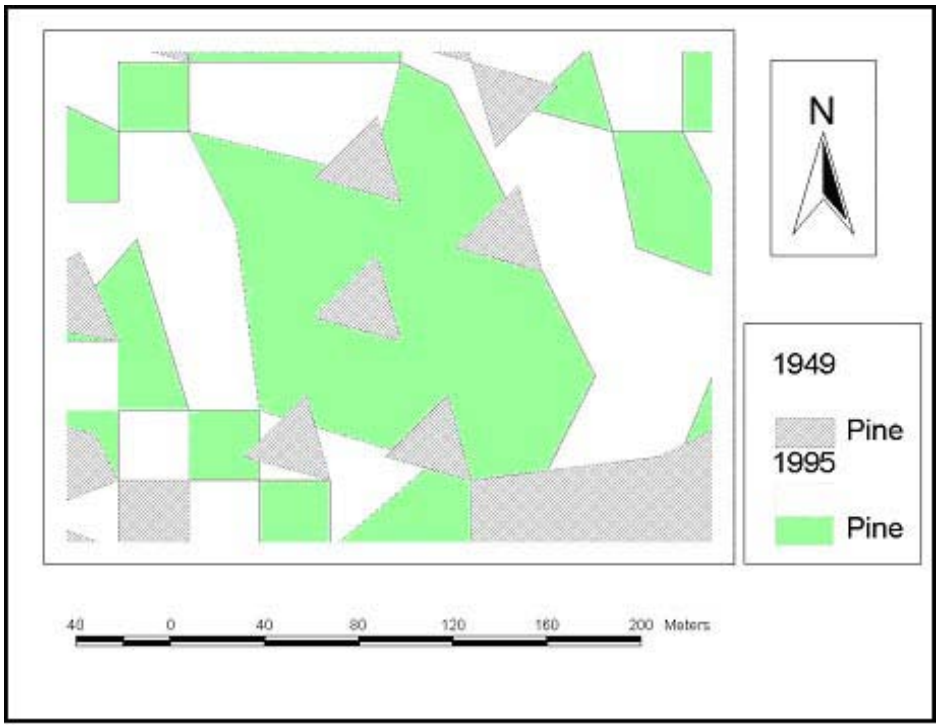

Fig. 5.4. Small *P. taeda* patches in 1949 grew and merged to form larger patches in 1995. This process showed significant changes of *P. taeda* patches even across a small time scale and accounted largely for the fewer but larger *P. taeda* patches in 1995 compared to 1949.

Other spatial statistical measures such as the edge metrics changed as well. Total edge (TE) increased by 28% from 566 kilometers (km) to 722 km (Table 5.3). Measures that reflect complexity showed some significant changes from 1949 to 1995 as well. Area-weighted mean patch fractal dimension (AWMPFD) increased from 1.23 to 1.32, and area-weighted mean shape index (AWMSI) increased from 6 to 14 indicating more complex landscape (Table 5.3). Both

AWMPFD and AWMSI are measures of shape complexity indicating that the shape of the pine patches changes from near regular to irregular.

Core metrics also changed between 1949 and 1995. Core area (NCA), total core area (TCA) and core area density (CAD) almost doubled from 1949 to 1995 (Table 5.3). Mean nearest neighbor (MNN) declined slightly from 35 to 33 m showing better connectivity or that the patches are closer to each other in a process of merging. For example, the largest pine stand in the two state parks in 1949 occupied 3.6% of the total area, but in 1995 it expanded to 8.8% as depicted by the largest patch index (LPI) in Table 5.3.

Results of soil analysis

Soils in the two state parks were found to be significantly different from the soils in Bastrop County. This difference hindered the comparison between the park pine distribution and the county pine distribution with respect to the distribution of soil attributes (Appendix D). Therefore, county and park levels were analyzed and reported separately.

At the county level, the distribution of *P. taeda* forests with respect to the soil distribution was non-random. *Pinus taeda* forests were over-represented on certain soils such as soils with higher sand content, lower clay content, lower

pH, higher permeability and lower fertility compared to the overall soil characteristics in the county. However, *P. taeda* forests thrived across wide range of soil characteristics.

With respect to clay content, the G-test was highly significant indicating that the distribution of *P. taeda* forests in Bastrop County was not random ($P < 0.001$, Table 5.4). The distribution of *P. taeda* forests in the county was not random as well with respect to drainage, slope, surface texture, CEC, organic matter, permeability and pH across the three different layers of soils at the 0.001 level of significance (Table 5.4). *Pinus taeda* in Bastrop County distributed randomly only in response to the bulk density and the pH in the first layer ($P>0.1$) (Table 5.4).

At the county level, *Pinus taeda* forests were commonly found on soils that contained low clay content. Almost 78% of *P. taeda* grew on soils that contained less than 29% clay (Fig. 5.5) such as sandy, loamy sand, sandy loam and sandy clay loam. Approximately 20% of *P. taeda* forests in Bastrop County grew on soils that contained more than 30-39% clay. Only 1% grew on clay content of more than 40% (Fig. 5.5).

Table 5.4

Results of the G-test show that *P. taeda* forests in Bastrop County are not randomly distributed with respect to distribution of soil attributes. The asterisk (*) indicates that where there was only one class, so no test could be made.

Soil variable	Layer	G-statistics	df	P-level
Drainage	-	13.32	2	<0.001
Slope	-	25.81	6	<0.001
Surface texture	-	55.44	12	<0.001
Bulk density	1	3.28	4	ns (>0.1)
	2	2.47	3	ns (>0.1)
	3	1.84	2	ns (>0.1)
Cation exchange capacity (CEC)	1	39.42	4	<0.001
	2	38.37	3	<0.001
	3	40.17	3	<0.001
Clay content	1	42.95	4	<0.001
	2	33.84	4	<0.001
	3	31.30	3	<0.001
Organic matter	1	21.21	3	<0.001
	2	*		
	3	*		
Permeability	1	23.7	3	<0.001
	2	43.1	3	<0.001
	3	18.16	2	<0.001
Soil reaction (pH)	1	1.82	3	ns (>0.1)
	2	8.00	3	<0.05
	3	53.26	3	<0.001

The importance of clay content in the distribution of *P. taeda* forests in Bastrop County is clear. Bastrop County soil distribution shows that 80% of the soils in the county contained less than 20% clay in the first layer (0-25 cm) and this is where 98% of *P. taeda* forests in the county grew (Table 5.5). In the second layer (25-86 cm), almost 72% of the soils in the county contain more than 20% clay and this is where 50% of *P. taeda* in the county grew (Table 5.5).

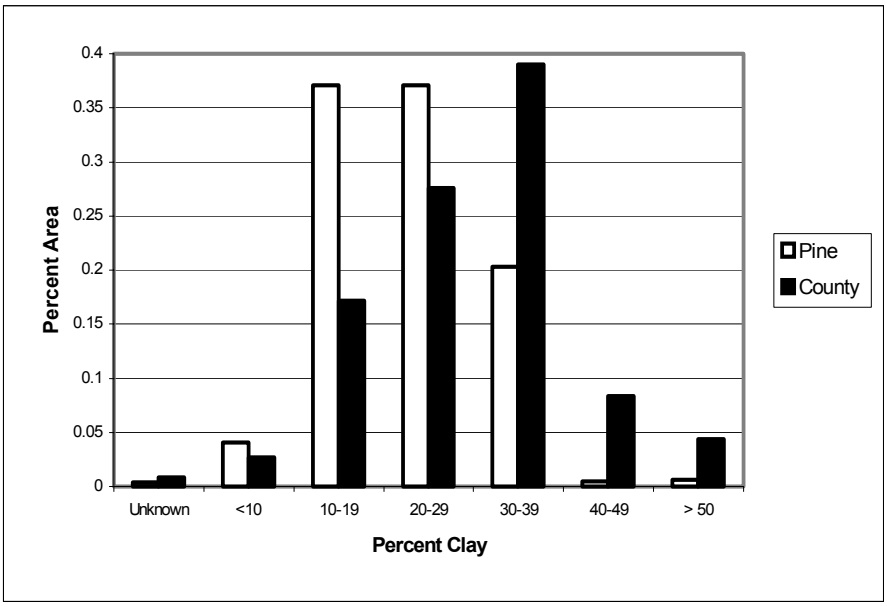

Fig. 5.5. Percent *P. taeda* area growing on different classes of soils with respect to clay content averaged over all layers. This was compared to the percent land area in the county with the same soil classes. *Pinus taeda* forests were overrepresented on soils with low clay content (< 29%) and underrepresented on soils with high clay content (> 30%).

Table 5.5

Distribution of the percentage of *P. taeda* area compared to total county area with different classes of clay content in different soil layers. *Pinus taeda* forests were overrepresented on soils with low clay content and underrepresented on soils with high clay content.

Layer	Average Depth (cm)	Clay content class (%)	Pine (%area)	County (%area)
1	0-25	Unknown	0.4	0.9
		< 10	60.4	30.9
		10-19	37.7	49.1
		20-29	0.5	6.6
		30-39	0.3	3.5
		40-49	0.1	4.4
		>50	0.6	4.6
		Sum	100	100
2	25-86	Unknown	0.4	0.9
		< 10	49.7	26.3
		10-19	0.5	1.4
		20-29	1.8	9.7
		30-39	0.2	0.02
		40-49	46.8	56.7
		>50	0.6	5.0
		Sum	100	100
3	85-140	Unknown	14.0	7.2
		20-29	40.3	21.8
		30-39	41.7	42.4
		40-49	3.4	24.2
		>50	0.6	4.4
		Sum	100	100
4	122-165	Unknown	49.2	43.3
		20-29	36.1	21.6
		30-39	14.2	25.7
		40-49	0.4	7.6
		>50	0.1	1.8
		Sum	100	100

Pinus taeda forests grew in areas with higher permeability. About 50% grew on soils with permeability of 53 mm/hr or more (Fig. 5.6).

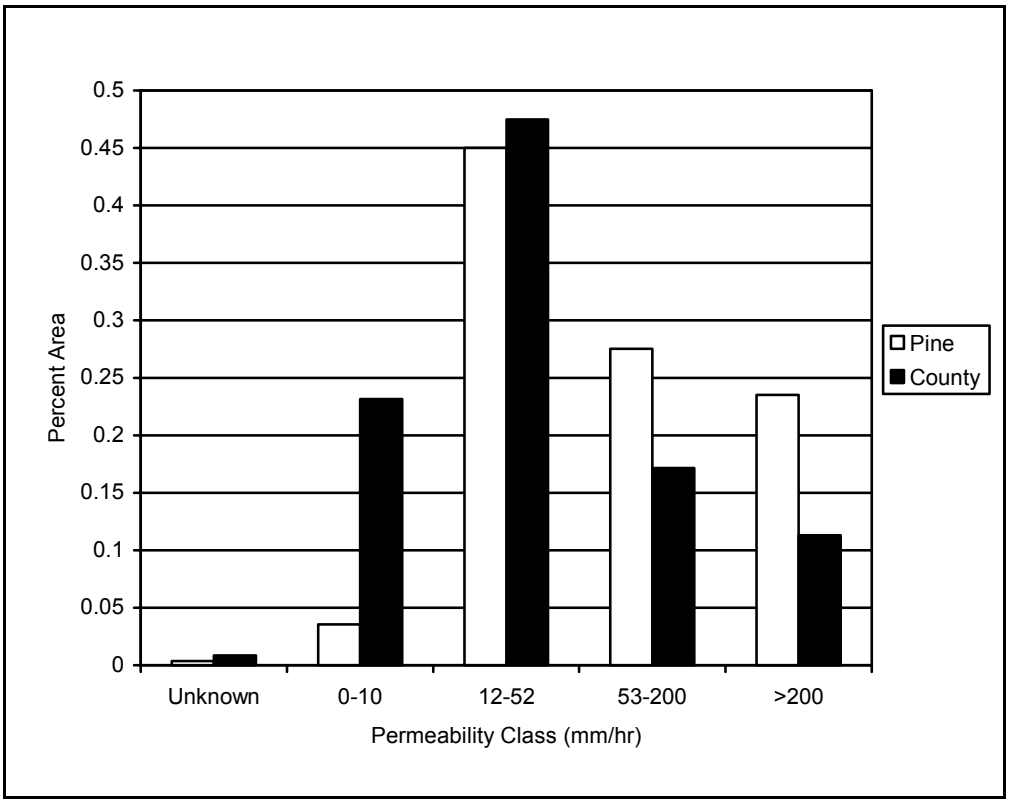

Fig. 5.6. Percent *P. taeda* area growing on different classes of soils with respect to permeability over all layers. This was compared to the percent land area in the county with the same classes. *Pinus taeda* forests were overrepresented on soils with high water permeability.

Pinus taeda forests also grew on soils with low fertility. More than 50% of *P. taeda* populations grew on soils with Cation Exchange Capacity of 5 to 10

meq/100g (Fig. 5.7). With respect to pH, *P. taeda* forests grew on soils with low pH. Approximately 75% of *P. taeda* forests grew on soils with a pH from 6.0 to 6.7 (Fig. 5.8).

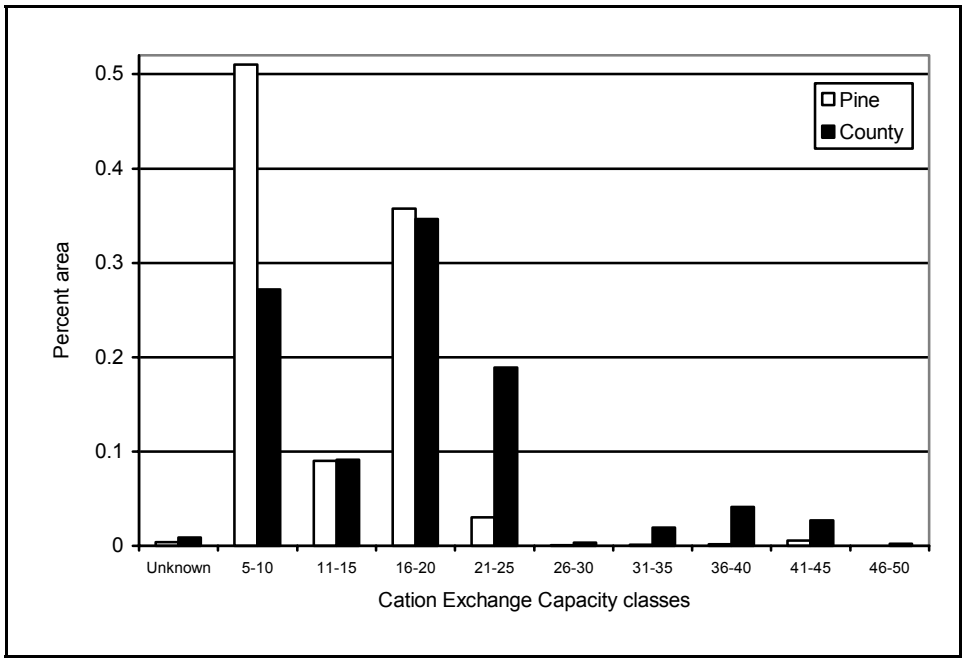

Fig. 5.7. Percent *P. taeda* area grew on different classes of soils with respect to Cation Exchange Capacity (CEC). This was compared to the percent land area in the county with the same classes. *Pinus taeda* forests were overrepresented on soils with low CEC.

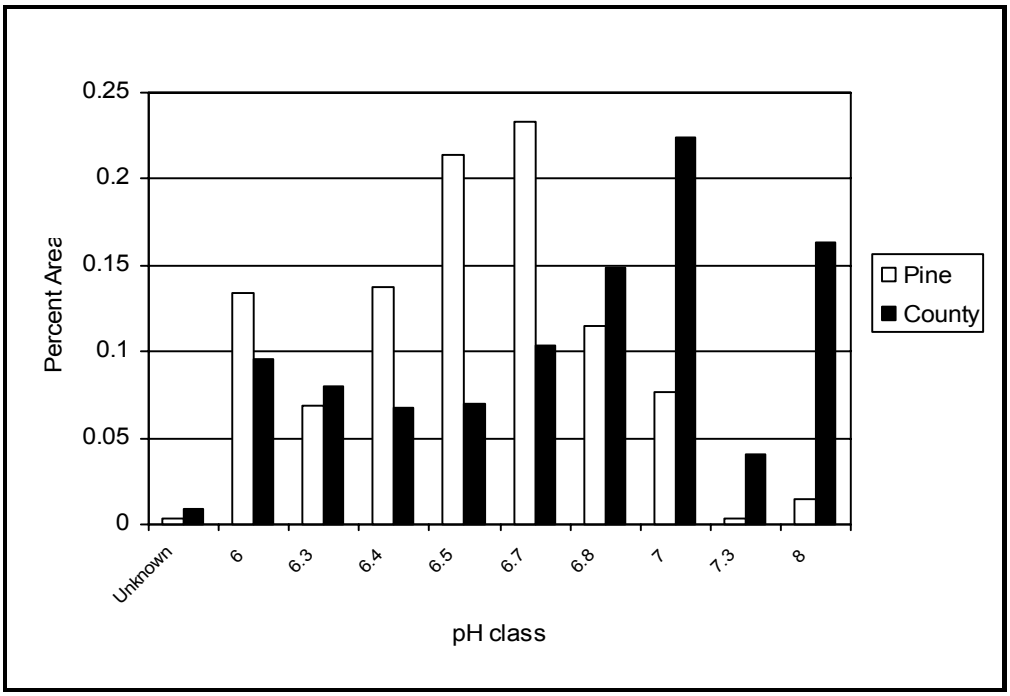

Fig. 5.8. Percent *P. taeda* area grew on different classes of soils with respect to pH. This was compared to the percent land area in the county with the same classes. *Pinus taeda* forests were overrepresented on soils with low pH and underrepresented on soils with high pH.

Weather results

The mean annual temperature for Bastrop fluctuated between 18.5 °C and 22.0 °C (Fig. 5.9) with a 60-year average of 20.1 °C. There were two periods when the temperature went above 20.5 °C, from 1941 to 1947 and from again 1996 to 2000 (Fig. 5.9). Precipitation fluctuated more dramatically than temperature

within the same time period with no apparent trend. The lowest precipitation in a year was 461 mm, and the highest was 1428 mm (Fig. 5.10).

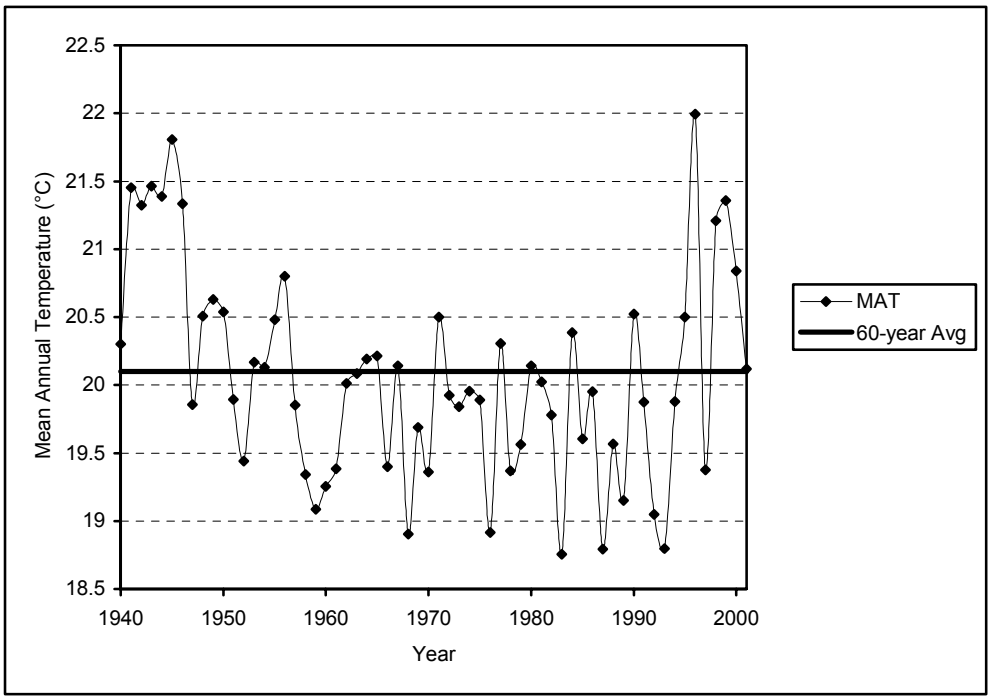

Fig. 5.9. Mean annual temperature (MAT) for Bastrop County showed a significant fluctuation during the past 60 years with mean annual temperature of 20.1 °C.

Weather parameters showed various degrees of fluctuation. Mean annual minimum temperature and mean annual maximum temperature showed the same trend as the mean annual temperature shown in Fig. 5.9 (see Appendix E). Total snowfall fluctuated from 0 cm/year to 15.24 cm/year over the period

from 1940 to 2001. There was a declining trend for snow events from 1986 to 2001 (Appendix E). Number of days with maximum temperature of 32.2 °C (90 °F) or more ranged from 79 to 208 days/year and showed a decline from 1940 to 1970 and then a steady increase from 1970 to 2001. Number of days with minimum temperature of 0 °C (32 °F) or less ranged from 7 to 59 days/year and showed a steady increase from 1940 to 1970 and then a decline from 1970 to 2001 (Appendix E).

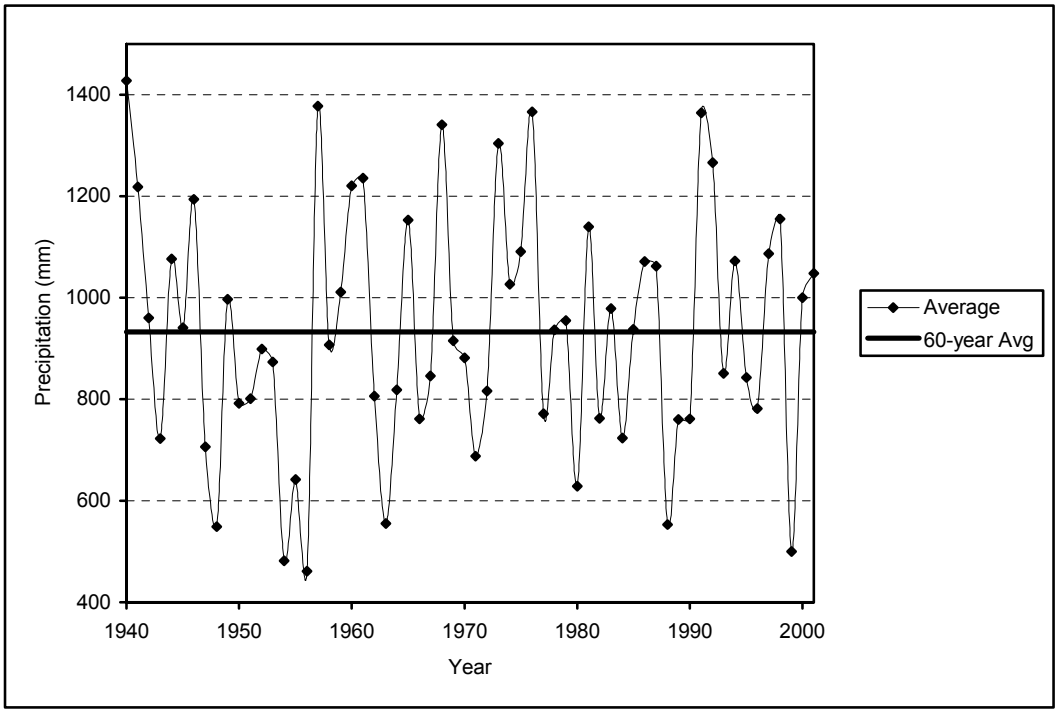

Fig. 5.10. Annual precipitation for Bastrop County showed a wide variability from one year to another with extreme dry and extreme wet years.

The number of days with precipitation of 25.4 mm or more averaged 11.4 days/year with a maximum of 19 days/year and a minimum of four days/year indicating that, on average, one fourth of the total rainfall in Bastrop County was received in 11 days of one calendar year. In other words, large amounts of rain fell over a short time interval. Number of days with a maximum temperature of 0 °C or less averaged 0.87 days/year and ranged from zero to six days/year. None of these weather elements showed any trend. There were great fluctuations from one year to the next in Bastrop County, the westernmost edge of *P. taeda* (Appendix E).

Discussion

Early aerial photographs opened a novel opportunity to explore the dynamics of the westernmost *P. taeda* population, the Lost Pines. Geographically referencing the aerial photographs and using overlapping areas between adjacent photographs made it possible to study vegetation dynamics over nearly half a century. The Lost Pines within the two state parks showed some interesting shifts in the absence of logging.

Lost Pines and logging

Reduced logging pressure especially in the two state parks and abandonment of agricultural fields appears to have prompted the recovery of the Lost Pines. The pine forest area increased and patch size expanded.

The fact that 36% of the pine patches in 1949 had disappeared by 1995 suggests that absence of fire for this period may have caused the dominance of hardwoods over pines. This should be taken in consideration for managing the two state parks to preserve these populations of *P. taeda*. Prescribed burning might help alleviate the impact of hardwoods and enhance the growth of pine stands.

Evidence for the movement of *P. taeda* into open areas inside and outside the two state parks was noticeable throughout the aerial photographs and an example is shown in Fig. (5.11).

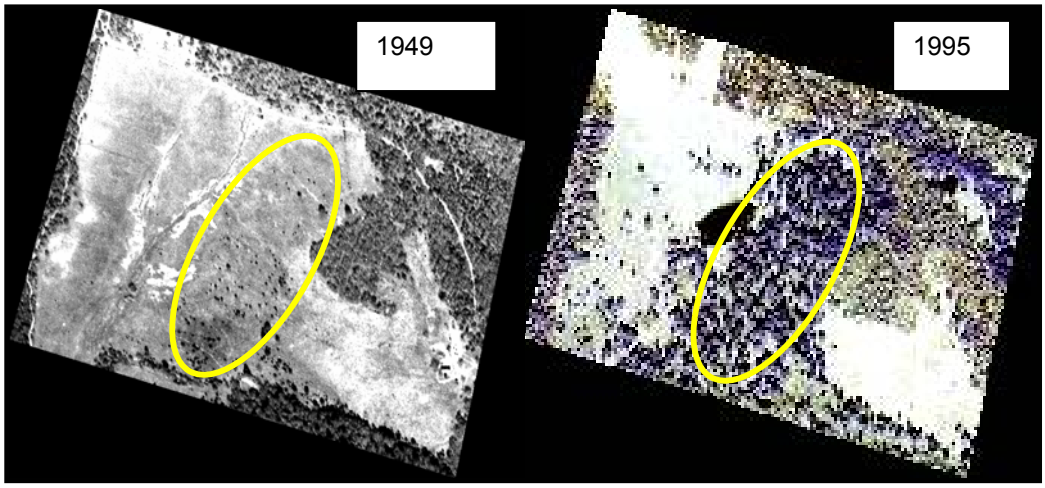

Fig. 5.11. Aerial photographs of Bastrop State Park in 1949 and 1995 showed an example of how *P. taeda* forest invaded an abandoned field.

The net outcome of 45 years of change was the increase in the pine patches in the two state parks, but stand dynamics were quite surprising. The newly formed patches can be explained to a great extent by small fires or an absence of logging. The availability of open areas for pine stands to establish must be explained by gaps left by death of older trees and small fires. The disappearance of pine patches over time may be the result of two hypothesized reasons: fire incidents or competition by hardwoods. To test these hypotheses, aerial photographs will be needed to track pine patches over shorter periods of time. With the absence of fire and disturbance for longer than 35 years, pine forests will gradually decline, giving way to late-succession hardwoods. Fire

suppression in the two state parks leads to severe competition by hardwoods and decline of the Lost Pines (Pyne et al., 1996; Wade et al., 2000).

Lost Pines and soil conditions

The distribution of the Lost Pines in relation to soil characteristics was complex. Results were not clear cut or definitive because soil types in the two state parks were not representative of the soil types in Bastrop County. The effect of soil characteristics can only be studied at the county level.

At the county level, *P. taeda* distribution was influenced by soil characteristics. *Pinus taeda* forests were prevalent on sandy topsoils over clayey sub-soils, well-drained soils, highly permeable soils and infertile soils. However, this distribution could be an artifact of the human preference for flat rich fertile agricultural soils and other anthropogenic activities such as grazing or housing development. These may be “leftover” soils in the area where the two state parks were established. Support for this view comes from the observation that in its natural range, *P. taeda* grows across various soil types and characteristics (Wahlenberg, 1960; Farmer, 1988; Shultz, 1997).

Another more likely explanation is that pines grow on these soils because the prevailing climatic conditions are so harsh. On average, one fourth of the

annual precipitation for Bastrop County can fall in approximately 11 days. Highly permeable and light-textured topsoils intercept torrential rainfall avoiding loss of runoff. The heavier sub-soil layers can retain this water over long periods of time.

Pinus taeda dynamics at the westernmost and disjunct population of the Lost Pines showed considerable dynamics over the past 45 years from 1949 until 1995. Pine patches increased in area and merged to form larger patches. Pine patches appeared and disappeared during this period due to the interaction of logging and fire. Fire suppression within the two state parks threatens the future of the Lost Pines population. *Pinus taeda* forests in Bastrop County appear to be restricted to specific soil characteristics with light sandy topsoils over clayey heavy subsoils due to prevalent harsh climatic conditions but *P. taeda* was found on other soil characteristics as well.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The use of interdisciplinary data sources in conjunction was powerful in understanding population dynamics on three time scales. The use of DNA markers with sound statistical analyses provided signatures of population dynamics on a geological time scale. Analysis of historical records combined with DNA fingerprinting showed the origin of the Lost Pines population. Spatial sciences showed dynamics at the recent time scale. Aerial photographs from 1949 and 1995 provided the source data for the spatial analysis and GIS modeling. DNA markers, remote sensing and GIS analysis combined with historical records and knowledge of the geological history built a working model for long-term management and forest preservation. The Lost Pines population is valuable for predicting climate changes on biota but it is also a local treasure with an uncertain future.

A hierarchical study with multiple time scales has the advantage of building and testing various hypotheses and models. Evidence for any model at any hierarchy can be obtained from the same level and from higher or lower levels of the hierarchy as well. For example, the steady-state hypothesis suggests that the Lost Pines may have been isolated for a long time because the pine forest in central Texas is constrained to a certain soil type over a geological

time scale. This model was not supported by genetic bottleneck tests. However, the observation that *Pinus taeda* is constrained to a certain soil type was supported when the pine-soil distribution was analyzed on a recent time scale using GIS analyses. Within Bastrop County, *P. taeda* forests are over-represented on certain soil types under harsh climatic conditions. The interaction of water level with these certain soil types remains to be determined.

Range-wide dynamics of the species at the geological time scale showed that populations east of the Mississippi River Valley are more genetically diverse than the populations west of the Mississippi River Valley. Genetic differentiation is modest yet significant, showing clear separation between eastern and western populations.

The two refugia model was supported as an explanation for the differentiation of eastern vs. western populations. However, the model predicts equal and comparable genetic diversity for both populations, but the eastern populations harbor higher levels of genetic diversity than the western populations. Knowledge of the life history characteristics and the geological history of the area explained the discrepancy in the prediction of this model. Being monoecious, long distance pollen movement combined with the prevailing wind direction from west to east (Bartlein et al., 1998) suggests unidirectional gene

flow. The predominately unidirectional long distance gene flow can explain the higher genetic diversity of the eastern populations. This also explained the higher private and diagnostic alleles in the eastern populations as well. The same observation was also made at a smaller spatial scale between the Lost Pines and the Western Gulf populations.

The two-refugia model is supported by pollen profile studies in central Texas which show that southern pines existed in the area at least 16,000 years ago (Bryant and Holloway, 1985). However, analyses of pollen profiles have their own drawbacks in describing the vegetation of a locality; still, they are widely used in studies of vegetation change. As a suggestion for future research, a phylogeographic approach with information on population haplotypes from nuclear and organelle DNA (such as mitochondrial DNA) should be conducted and then analyzed using GIS and geostatistics.

The two-refugia model of Wells et al. (1991) hypothesized the location of the two refugial populations east and west of the Mississippi River Valley. Fig. (11) in Wells et al., (1991) showed their hypothesized refugia during the Pleistocene with the Lost Pines population as part of the western refugia. If true, then genetic diversity of the Lost Pines might have been higher than the genetic diversity observed at the present time. The diversity may have been reduced by the bottleneck later on. This model needs further testing.

The analysis of single-locus allelic forms and multi-locus allelic forms in the Lost Pines suggested their close similarity to the Western Gulf population, especially the southwestern region, implying that these populations share a similar ancestry. This similarity refutes the idea that this population is a recent introduction from eastern sources (Youngman, 1965). These results are also supported by fossil pollen in central Texas assuming that pollen is coming from local *P. taeda*. Our DNA-based results support historical records.

Limiting factors for *Pinus taeda* population expansion in the westward direction are mainly precipitation and temperature (Hocker, 1956; Manogaran, 1975). Prevalent climatic conditions in central Texas 11,000 to 21,000 years ago are believed to have been more mesic than current conditions (Toomey et al., 1993). Consequently, pines forests may have grown southward and westward into the Edwards Plateau as suggested by Sorenson et al. (1972).

Soil characteristics are not a deterrent to pine forest expansion if climatic conditions are mild. *Pinus taeda* grows on a wide range of soil types where climatic conditions were near optimum (Wahlenberg, 1960; Baker and Langdon, 1990). Nonetheless, soil characteristics for *P. taeda* become more limiting factors when climatic conditions change to droughty. The observation that the pines are constrained to certain soil type may be correct at the western

edge of the species where climatic conditions are limiting but the experimental design did not yield clear cut and definitive answers. The Lost Pines tend to grow on light topsoils with heavier sub-soils. These soils may capture the torrential rain. The heavy sub-soils hold the moisture, releasing it as needed to support forest survival in extreme years.

The shift in climate to sub-optimal conditions for pines is believed to have culminated in the middle Holocene, 5,000-2,500 years ago. A set of population contractions was hypothesized for this time period. Support for a population contraction came from a significant genetic signature of one or more bottleneck events in the Lost Pines. High levels of genetic diversity despite the bottleneck may have been the paradoxical result of post-bottleneck expansion of the Lost Pines. The geological history of this area suggests that there was a period of climatic relief 2,500 to 1,500 years ago (Toomey et al., 1993). This period may have contributed to the expansion of the Lost Pines by merging patches. A genetic bottleneck signature is less likely to have come from the last interglacial period (100,000 – 70,000 YBP) (Emiliani, 1971) than a more recent bottleneck event.

Aerial photographs are valuable resources for tracking vegetation or species dynamics (Hudak and Wessman, 1998; Kadmon and Harari-Kremer, 1999; Taylor et al., 2000; Petit and Lambin, 2001). The use of these aerial

photographs was accelerated by the utilization of overlapping areas between photographs to facilitate the georeferencing.

The future of the Lost Pines is uncertain. Fire suppression may be a factor for the disappearance of some pine patches in the two state parks. Future research should be directed toward the study of annual changes of pine patches within the two state parks and relate this to the available history of the two parks of any fire or logging events. Such studies can provide more detailed insights to the dynamics of the Lost Pines. Similarly, these studies should be directed to determine the relationship of *P. taeda* and other forest species to the forest management regime within the state parks. Local silvicultural experiments can also provide information about the survivability of the pine seedlings under various management regimes.

The Lost Pines apparently survived long history of climate change from mild to harsh. It also survived periodical logging and fire. The efforts should be directed to enhance and preserve these relicts of pine stands for the future time scale and future generations.

The preservation of the Lost Pines can provide a long term study site where relationships between climate change and ecosystem dynamics can be more thoroughly explored.

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APPENDIX A

The following is an isozyme data for *Pinus taeda* presented by Florence and Rink (1979). This data might be hard to locate. They are useful for comparison purposes in this study and for future studies. These data corresponds to the data presented in Table (2) of Florence and Rink (1979).

TXC = Central Texas

TXE = East Texas

AR = Arkansas

LA = Louisiana

MS = Mississippi

AL = Alabama

GA = Georgia

SC = South Carolina

N^a = Sample size

MNA = Mean number of allele per locus. MNA (the last row of this table) have been calculated from their data but they have not calculated it themselves in their study.

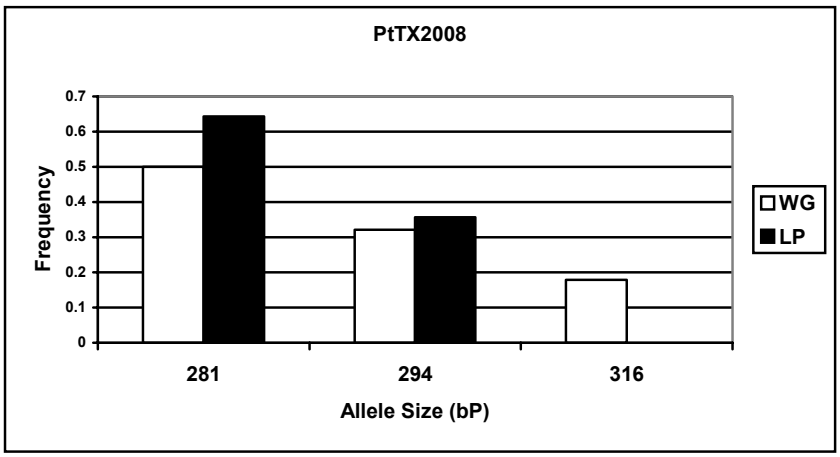
Table A.1

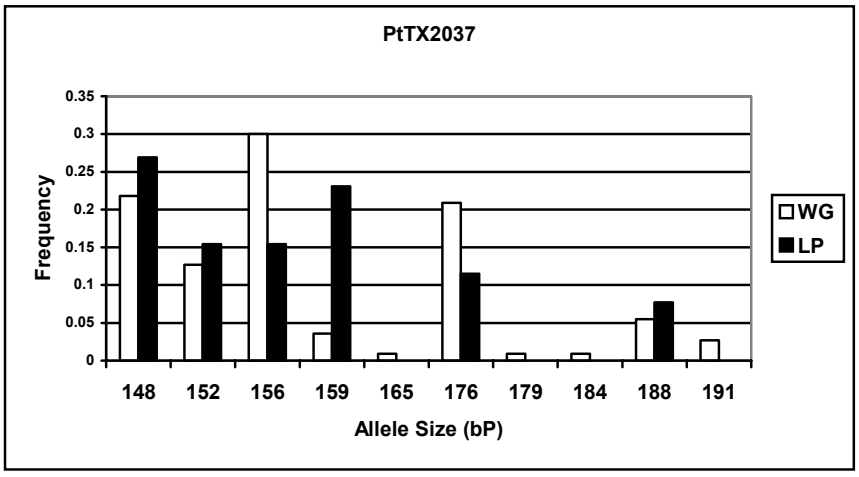
Florence and Rink (1979) isozyme data for *P. taeda* populations.

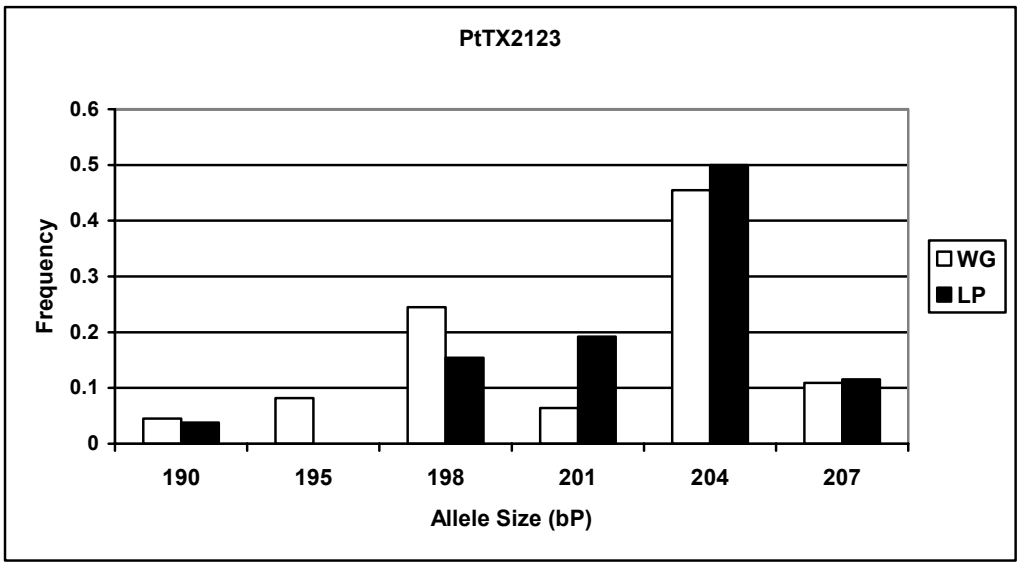
Locus	Allele	TXC	TXE	LA	AR	MS	AL	GA	SC	Overall Freq.
AcP	1	0	0	0	0	0.38	0	0.017	0.111	0.015
	2	0.308	0.354	0.150	0.067	0.238	0.250	0.133	0.278	0.248
	3	0.654	0.594	0.650	0.833	0.676	0.562	0.667	0.556	0.646
	4	0.038	0.021	0.050	0	0.038	0	0	0.056	0.024
	5	0	0.031	0.150	0.100	0.010	0.188	0.183	0	0.067
GDH	1	0.987	0.906	1.000	0.967	0.971	0.979	0.817	1.000	0.943
	2	0	0.021	0	0	0.010	0	0.033	0	0.011
	3	0.013	0.073	0	0.033	0.019	0.021	0.117	0	0.042
	4	0	0	0	0	0	0	0.033	0	0.004
GOT-A	1	0	0	0	0	0.011	0.062	0.083	0	0.020
	2	0.218	0.065	0.400	0.300	0.130	0.188	0.300	0.222	0.246
	3	0.782	0.635	0.600	0.700	0.859	0.750	0.617	0.778	0.734
GOT-B	1	0.038	0.167	0.150	0.100	0.087	0.062	0.133	0.056	0.099
	2	0.962	0.812	0.850	0.900	0.913	0.938	0.833	0.889	0.890
	3	0	0.021	0	0	0	0	0.033	0.055	0.011
GPI	1	1.000	0.990	1.000	0.967	1.000	0.979	0.983	0.944	0.989
	2	0	0.010	0	0.033	0	0.021	0.017	0.056	0.011
MDH-A	1	0	0	0	0.033	0	0	0	0	0.002
	2	0.974	0.969	0.850	0.934	0.990	0.938	0.950	0.944	0.961
	3	0.026	0.031	0.150	0.033	0.010	0.042	0.033	0.056	0.033
	4	0	0	0	0	0	0.020	0.017	0	0.004
MDH-B	1	0.731	0.667	0.750	0.833	0.457	0.562	0.533	0.389	0.604
	2	0.244	0.281	0.150	0.167	0.476	0.417	0.467	0.611	0.358
	3	0.013	0.031	0.100	0	0.067	0.021	0	0	0.031
	4	0.012	0.021	0	0	0	0	0	0	0.007
MDH-C	1	0	0	0	0	0	0.021	0.017	0	0.004
	2	1.000	1.000	1.000	1.000	1.000	0.979	0.983	1.000	0.996
6-PGD-A	1	0	0.011	0	0	0	0	0.050	0.111	0.013
	2	0.397	0.375	0.400	0.400	0.419	0.500	0.233	0.278	0.367
	3	0.029	0.023	0	0.067	0.181	0.042	0.233	0	0.090
	4	0	0.023	0	0	0.029	0.062	0.017	0	0.018
	5	0	0	0	0	0	0	0	0.111	0.004
	6	0.574	0.568	0.600	0.533	0.371	0.396	0.467	0.500	0.508
6-PGD-B	1	0.897	0.875	0.900	0.900	0.959	0.979	0.717	0.944	0.897
	2	0.013	0.031	0	0	0.027	0	0.267	0	0.048
	3	0.090	0.094	0.100	0.100	0.014	0.021	0.016	0.056	0.055
N[a]		39	48	15	10	>30	24	30	9	>205
MNA		2.3	2.9	2.0	2.2	2.7	2.6	3.1	2.3	

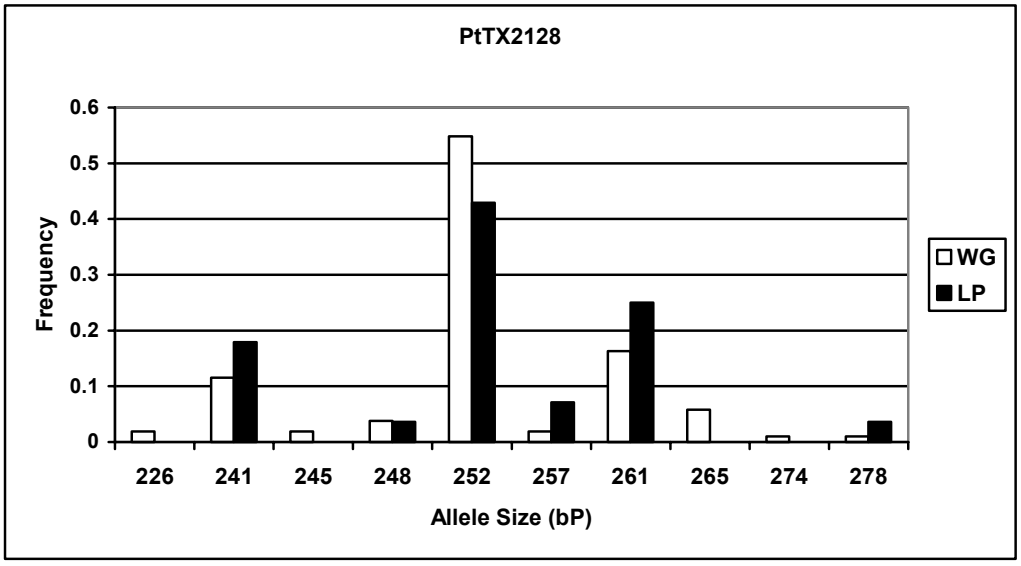
APPENDIX B

These are the frequency histograms of the various allelic sizes at the 34 microsatellite loci used. WG stands for Western Gulf; LP stands for Lost Pines.






PtTX2123
Frequency
0.6
0.5
0.4
0.3
0.2
0.1
0
190
195
198
201
204
207
Allele Size (bP)
WG
LP


PtTX2128
Frequency
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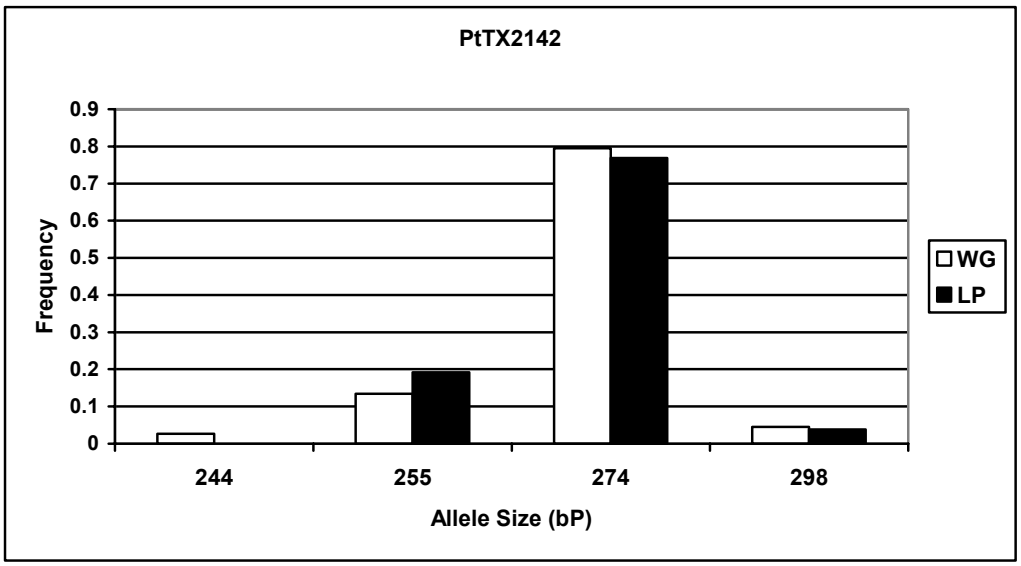
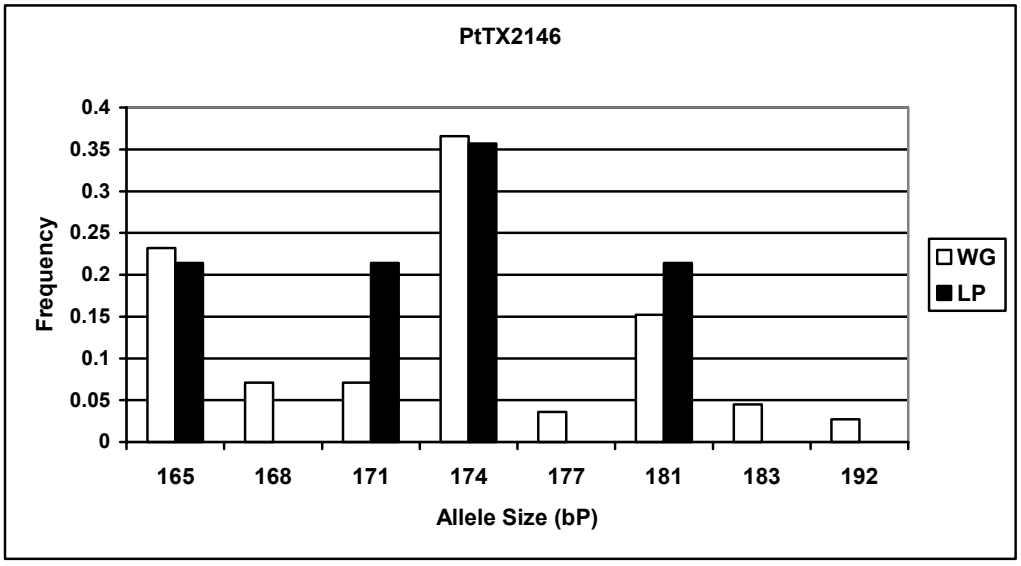
0.5
0.4
0.3
0.2
0.1
0
226
241
245
248
252
257
261
265
274
278
Allele Size (bP)
WG
LP


PtTX2142
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency
244
255
274
298
Allele Size (bP)
WG
LP


PtTX2146
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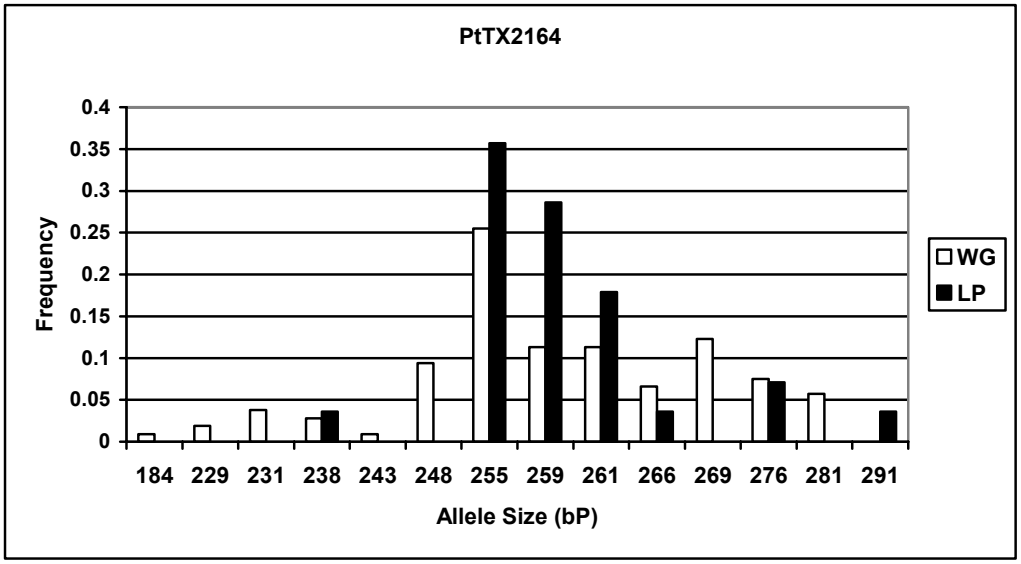
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
165
168
171
174
177
181
183
192
Allele Size (bP)
WG
LP


PtTX2164
Frequency
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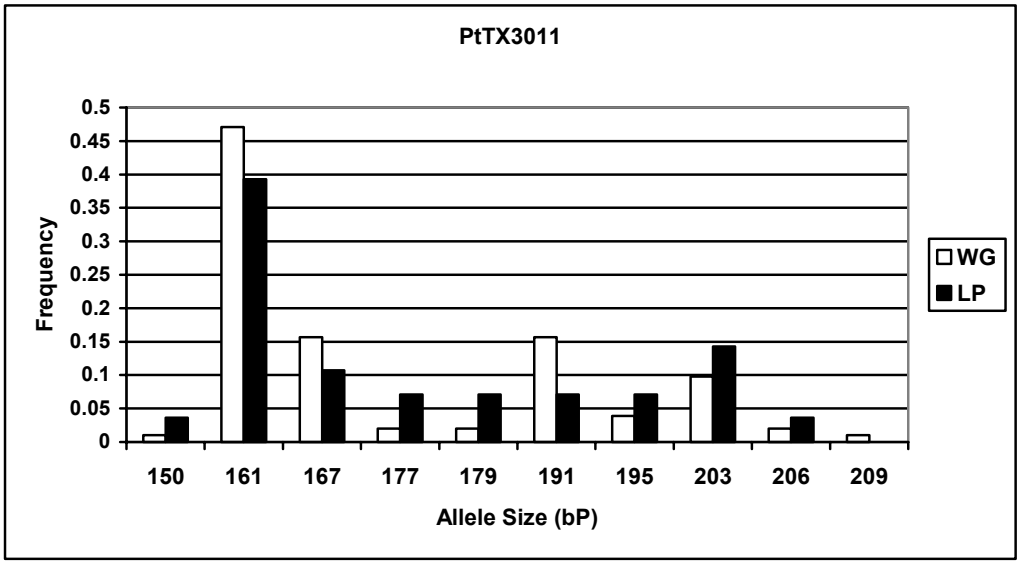
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
184 229 231 238 243 248 255 259 261 266 269 276 281 291
Allele Size (bP)
WG
LP


PtTX3011
Frequency
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0.45
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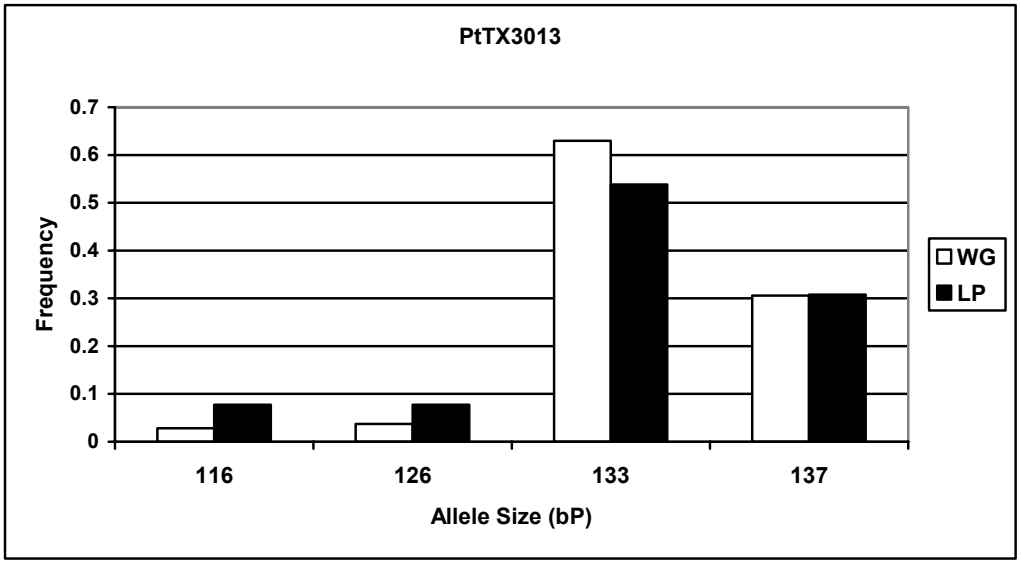
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
150 161 167 177 179 191 195 203 206 209
Allele Size (bP)
WG
LP


PtTX3013
Frequency
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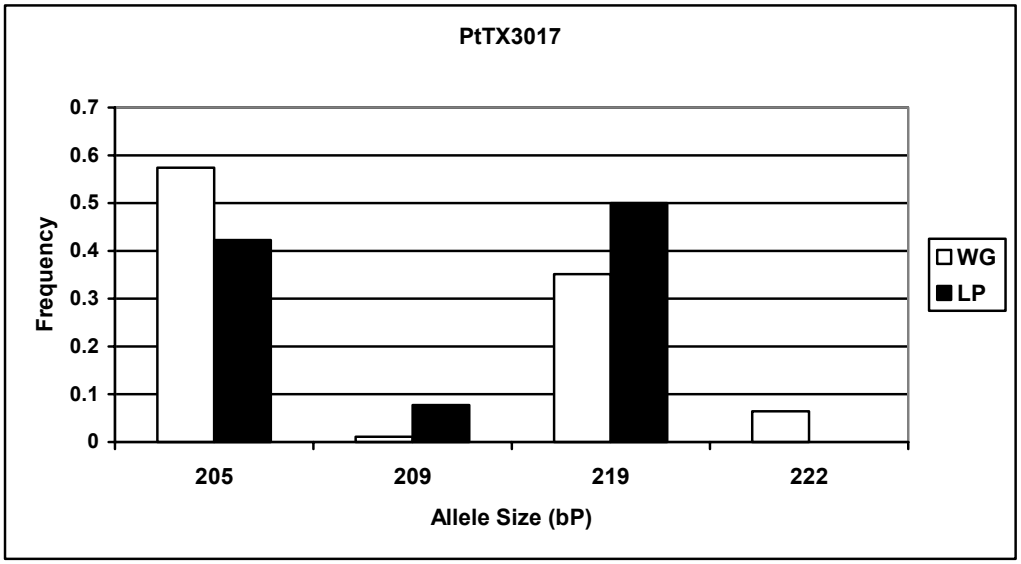
0.5
0.4
0.3
0.2
0.1
0
116
126
133
137
Allele Size (bP)
WG
LP


PtTX3017
Frequency
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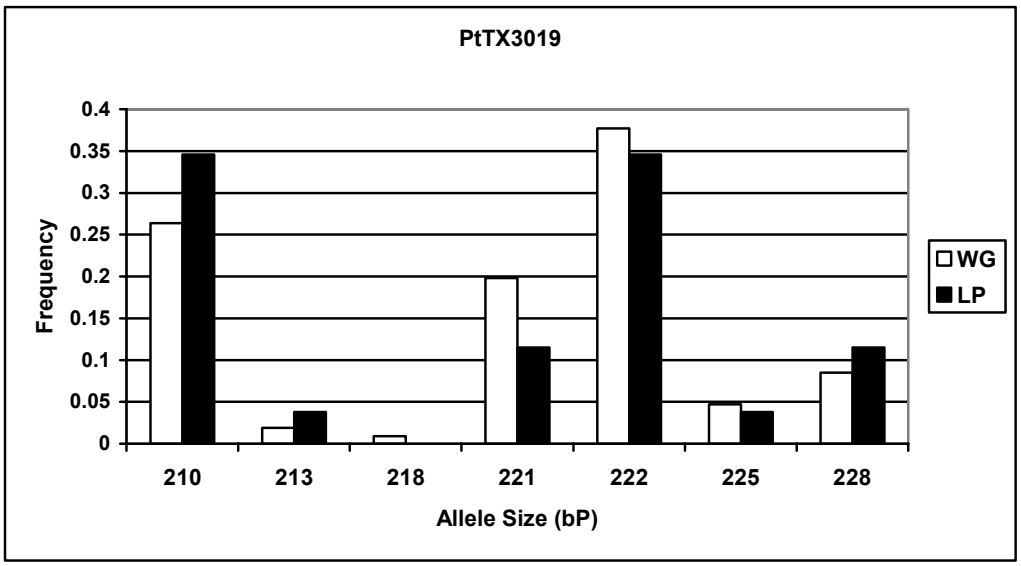
0.5
0.4
0.3
0.2
0.1
0
205
209
219
222
Allele Size (bP)
WG
LP


PtTX3019
Frequency
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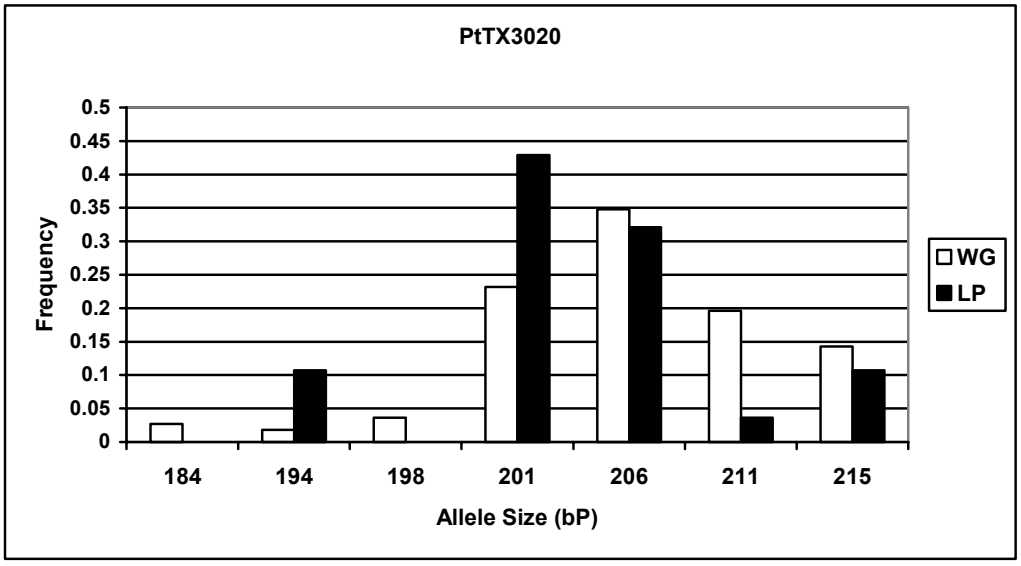
0.3
0.25
0.2
0.15
0.1
0.05
0
210
213
218
221
222
225
228
Allele Size (bP)
WG
LP


PtTX3020
Frequency
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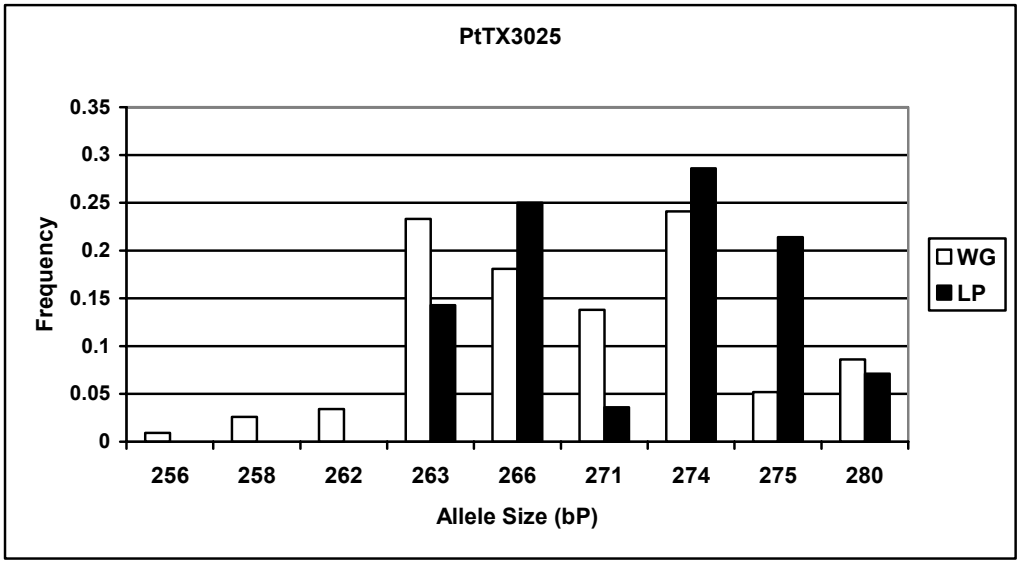
0.3
0.25
0.2
0.15
0.1
0.05
0
184
194
198
201
206
211
215
Allele Size (bP)
WG
LP


PtTX3025
Frequency
0.35
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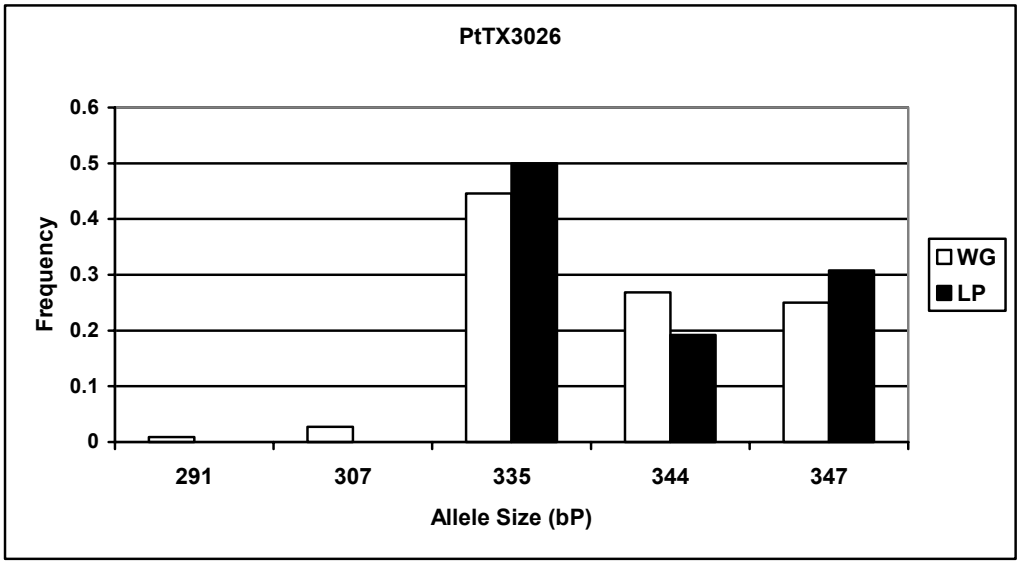
0.25
0.2
0.15
0.1
0.05
0
256
258
262
263
266
271
274
275
280
Allele Size (bP)
WG
LP


PtTX3026
Frequency
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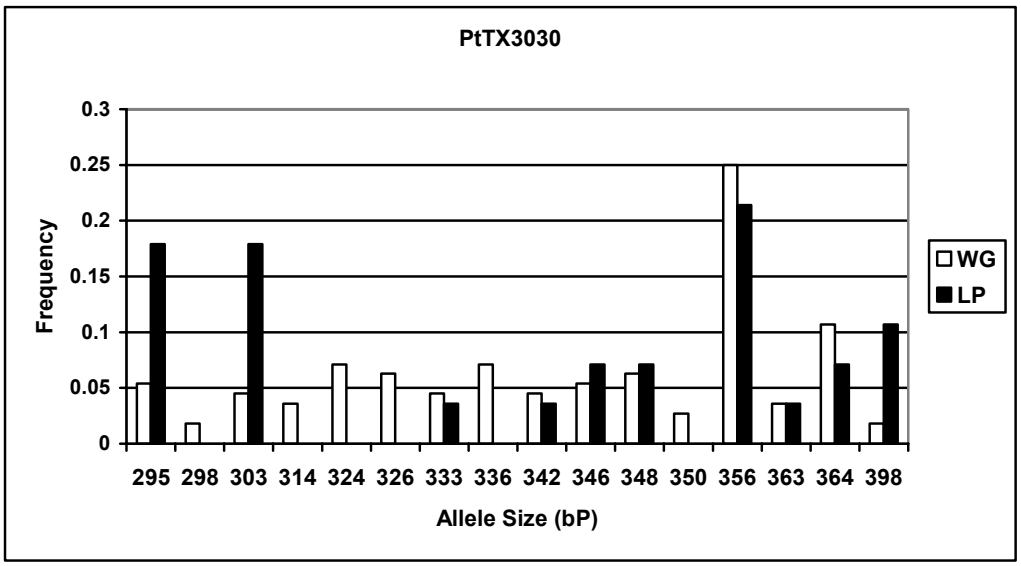
0.4
0.3
0.2
0.1
0
291
307
335
344
347
Allele Size (bP)
WG
LP


PtTX3030
Frequency
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0.1
0.05
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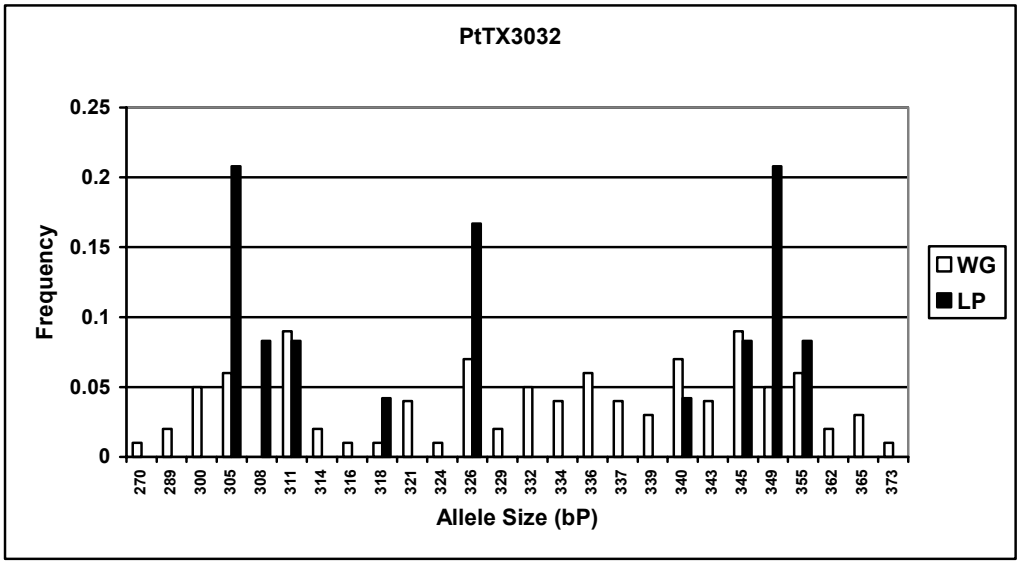
295 298 303 314 324 326 333 336 342 346 348 350 356 363 364 398
Allele Size (bP)
WG
LP


PtTX3032
Frequency
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0.15
0.1
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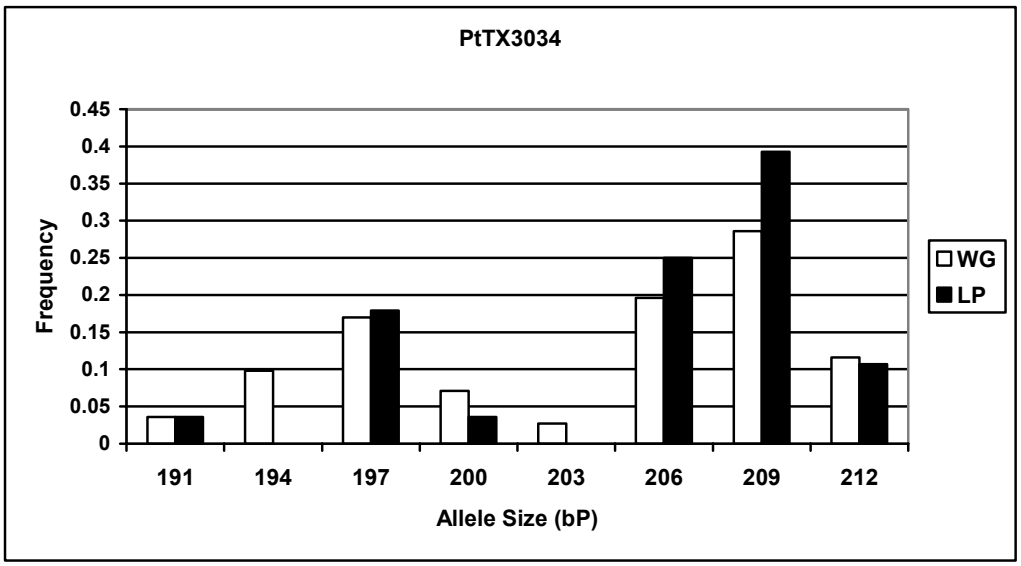
0
270 289 300 305 308 311 314 316 318 321 324 326 329 332 334 336 337 339 340 343 345 349 355 362 365 373
Allele Size (bP)
WG
LP


PtTX3034
Frequency
0.45
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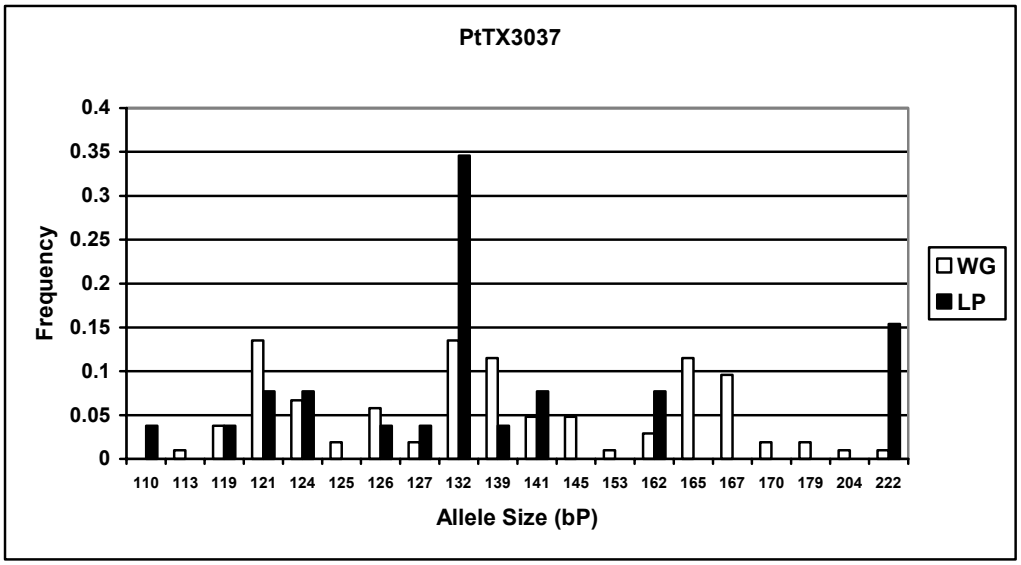
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
191
194
197
200
203
206
209
212
Allele Size (bP)
WG
LP


PtTX3037
Frequency
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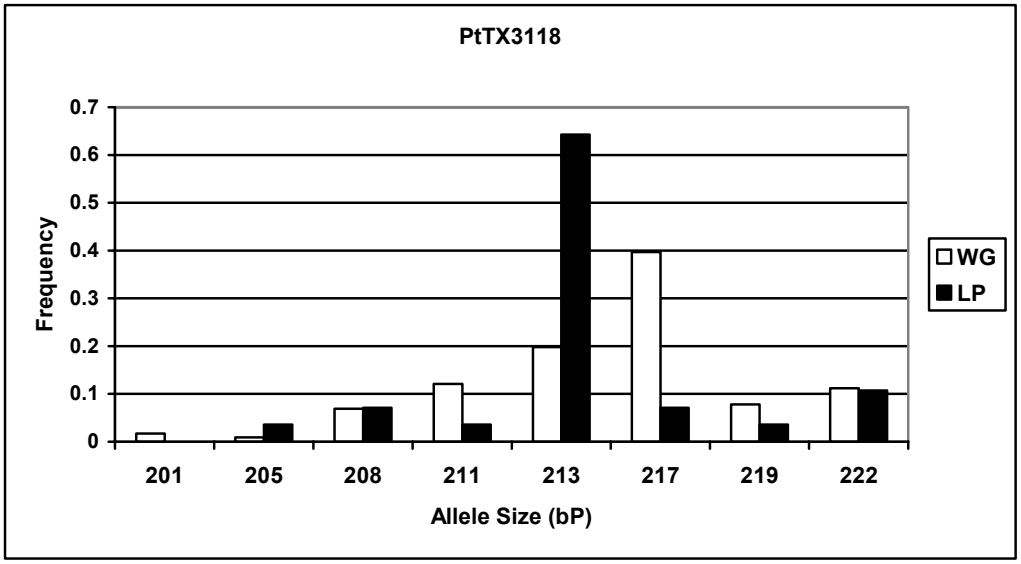
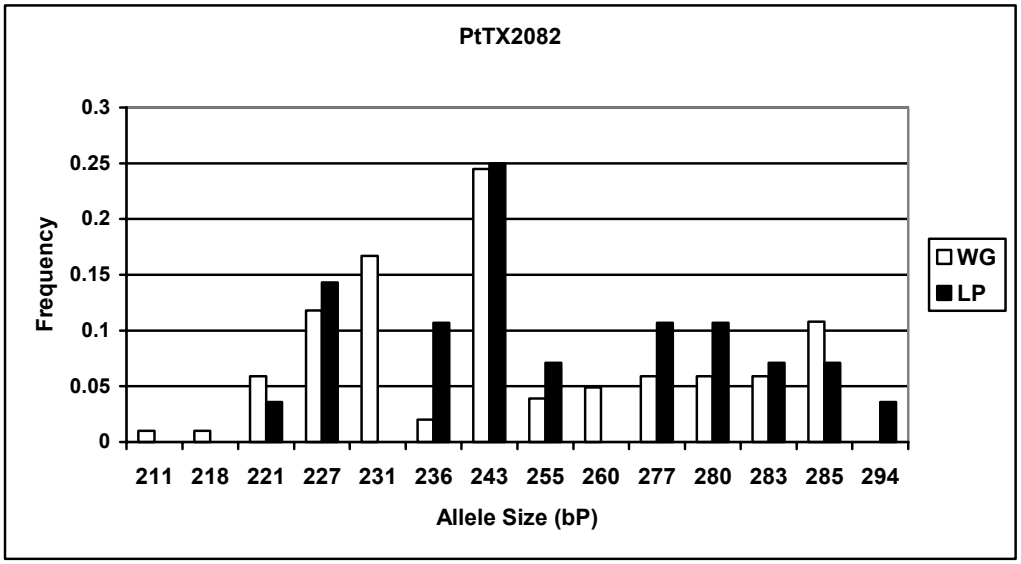
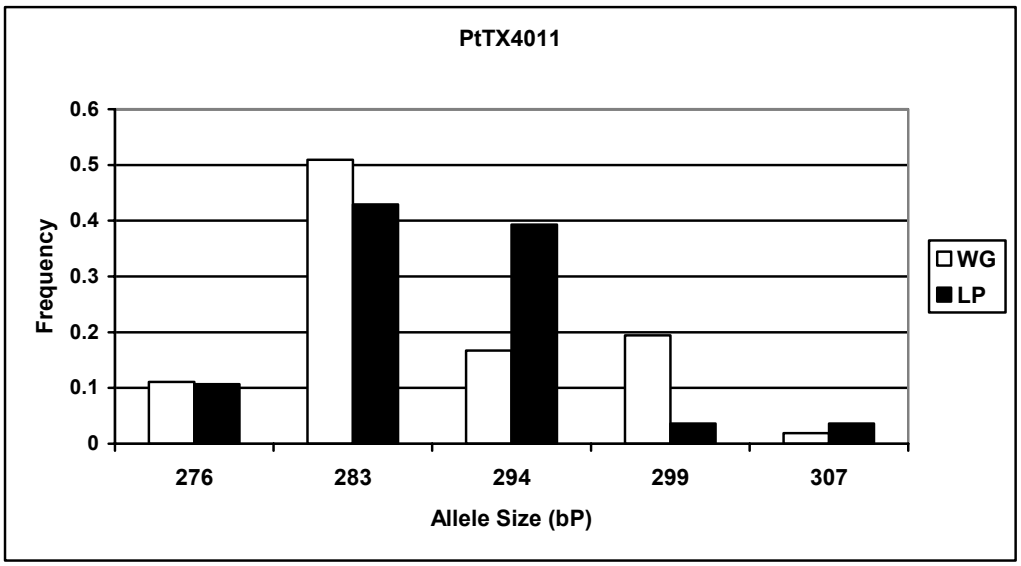
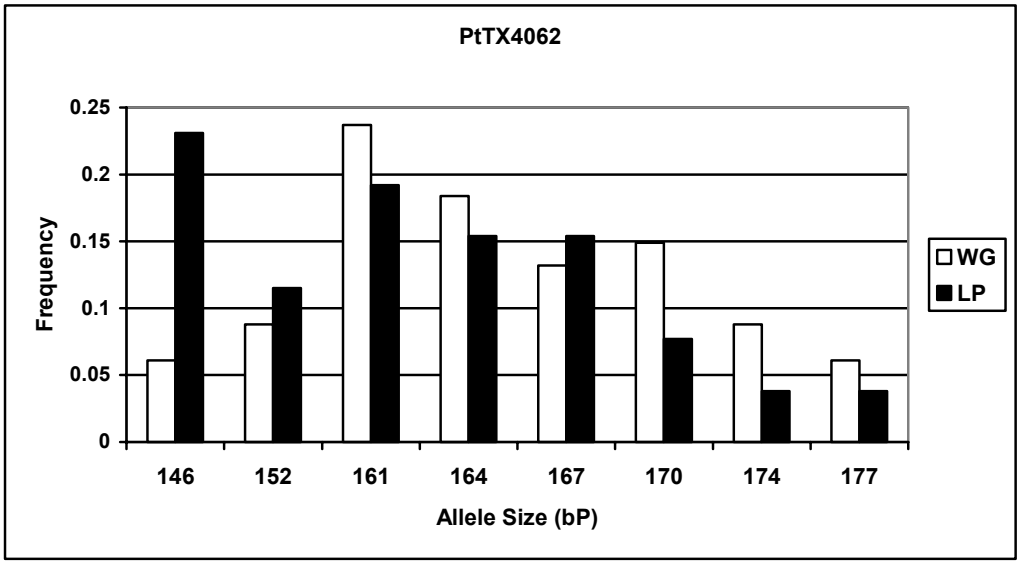
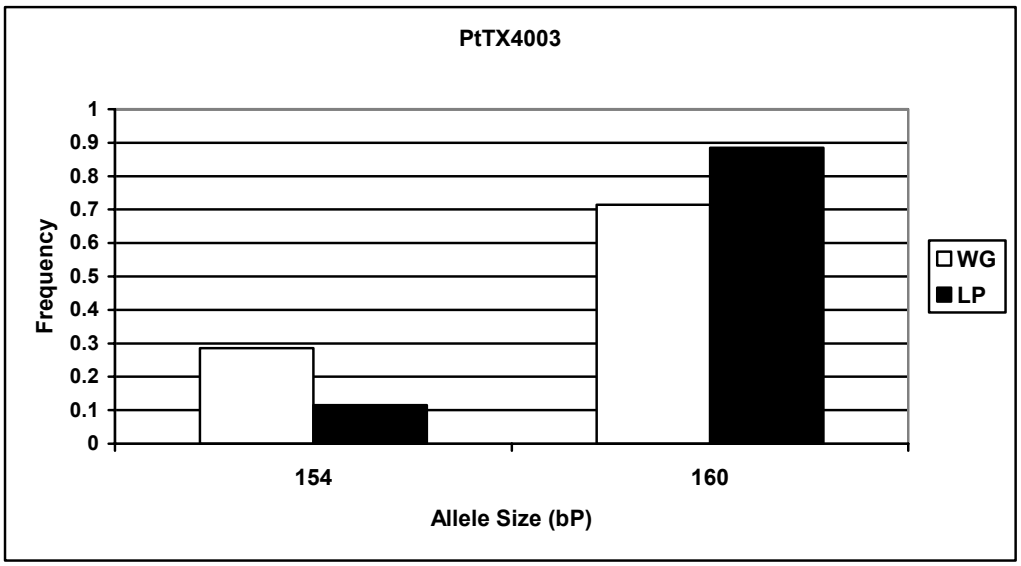
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
110 113 119 121 124 125 126 127 132 139 141 145 153 162 165 167 170 179 204 222
Allele Size (bP)
WG
LP


PtTX3118
Frequency
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
201
205
208
211
213
217
219
222
Allele Size (bP)
WG
LP


PtTX2082
Frequency
0.3
0.25
0.2
0.15
0.1
0.05
0
211
218
221
227
231
236
243
255
260
277
280
283
285
294
Allele Size (bP)
WG
LP


PtTX4011
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency
276
283
294
299
307
Allele Size (bP)
WG
LP


PtTX4062
0.25
0.2
0.15
0.1
0.05
0
Frequency
146
152
161
164
167
170
174
177
Allele Size (bP)
WG
LP


PtTX4003
Frequency
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0.9
0.8
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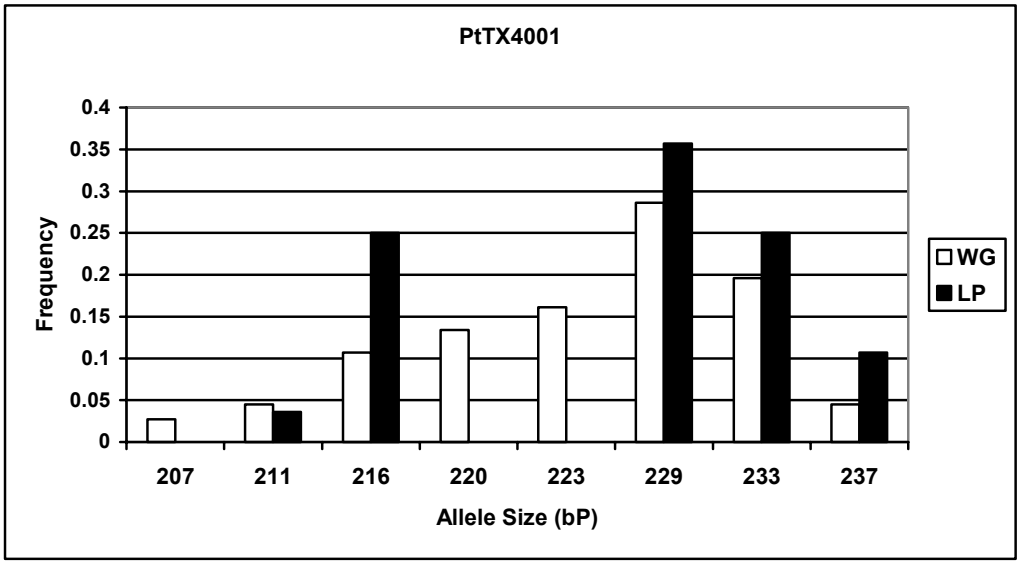
0.6
0.5
0.4
0.3
0.2
0.1
0
154
160
Allele Size (bP)
WG
LP


PtTX4001
Frequency
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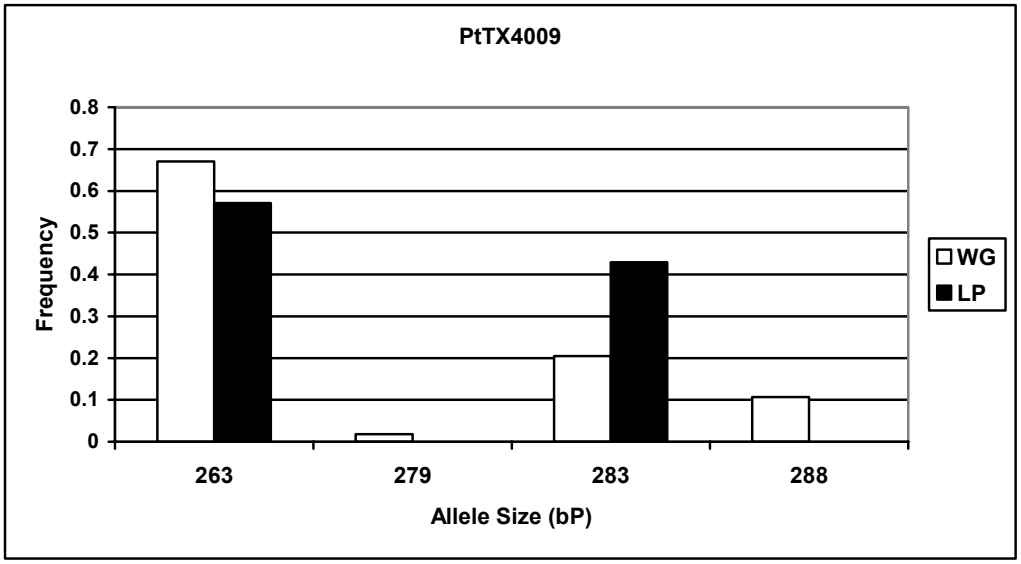
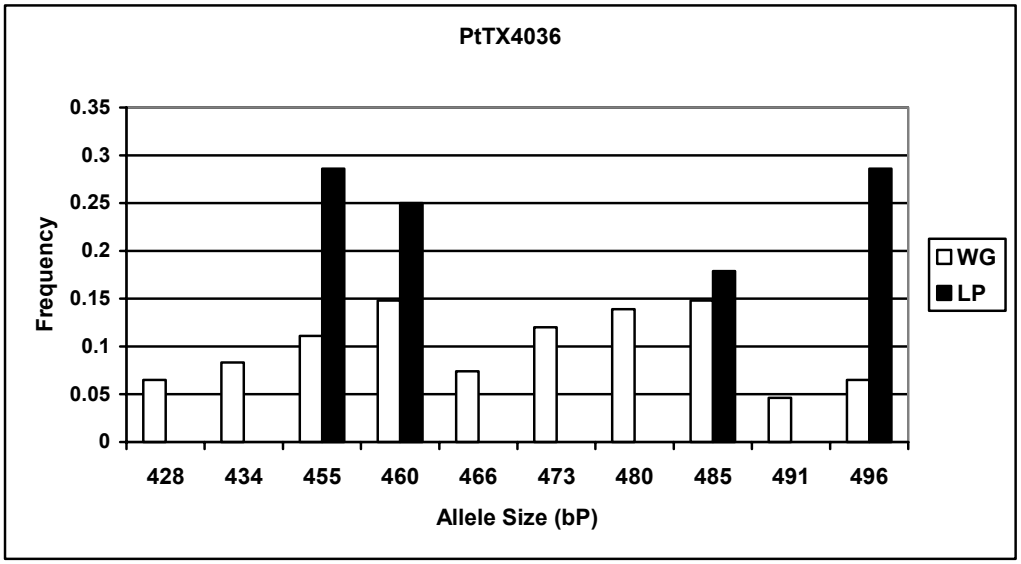
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
207
211
216
220
223
229
233
237
Allele Size (bP)
WG
LP


PtTX4009
Frequency
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
263
279
283
288
Allele Size (bP)
WG
LP


PtTX4036
Frequency
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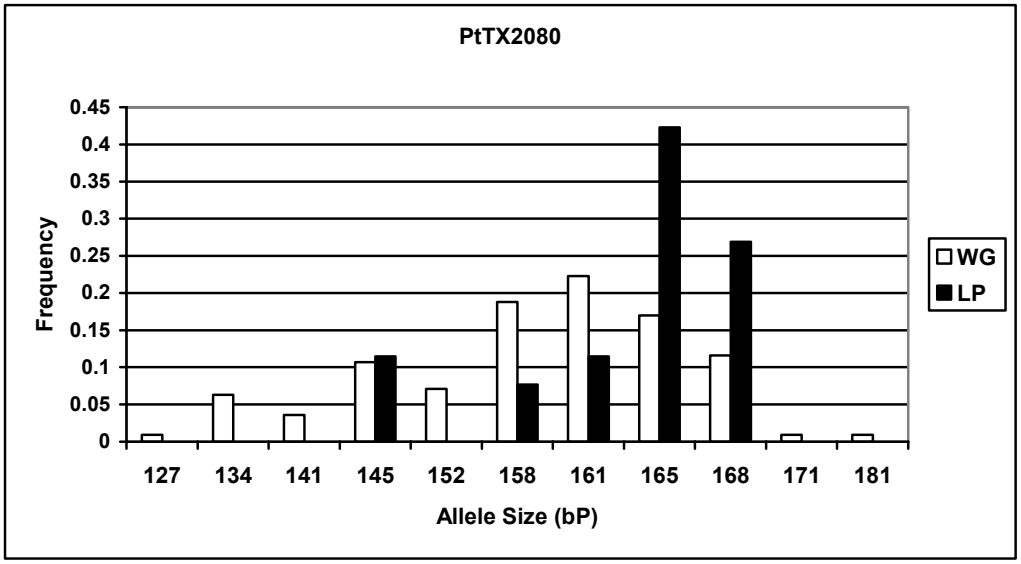
0.3
0.25
0.2
0.15
0.1
0.05
0
428
434
455
460
466
473
480
485
491
496
Allele Size (bP)
WG
LP


PtTX2080
Frequency
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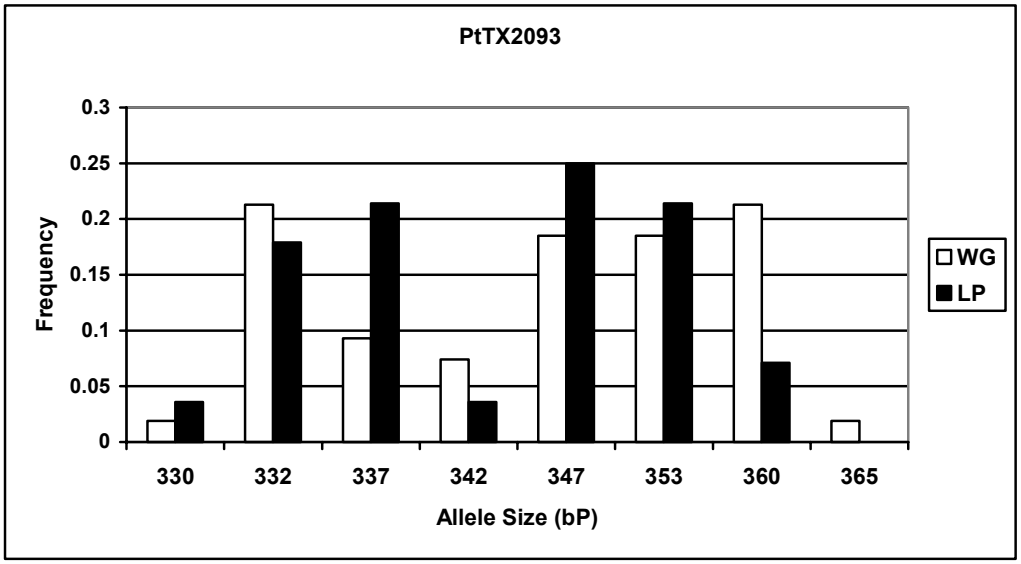
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
127
134
141
145
152
158
161
165
168
171
181
Allele Size (bP)
WG
LP


PtTX2093
Frequency
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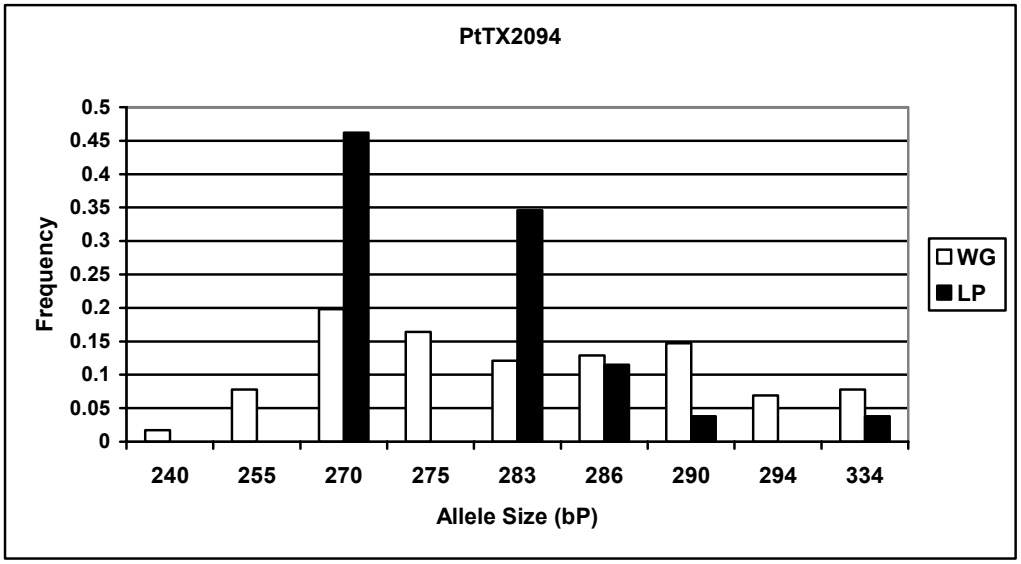
0.25
0.2
0.15
0.1
0.05
0
330
332
337
342
347
353
360
365
Allele Size (bP)
WG
LP


PtTX2094
Frequency
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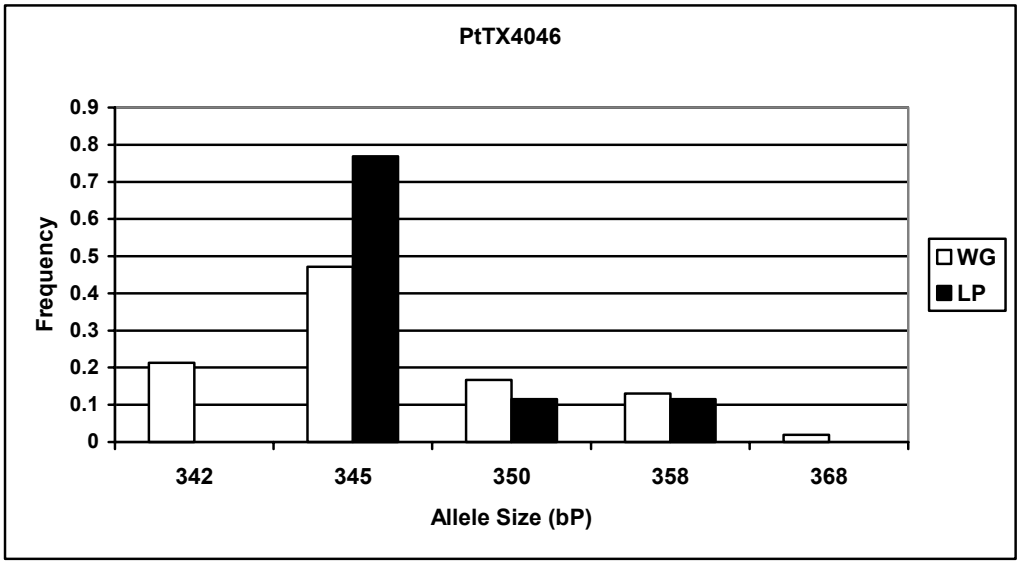
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
240
255
270
275
283
286
290
294
334
Allele Size (bP)
WG
LP


PtTX4046
Frequency
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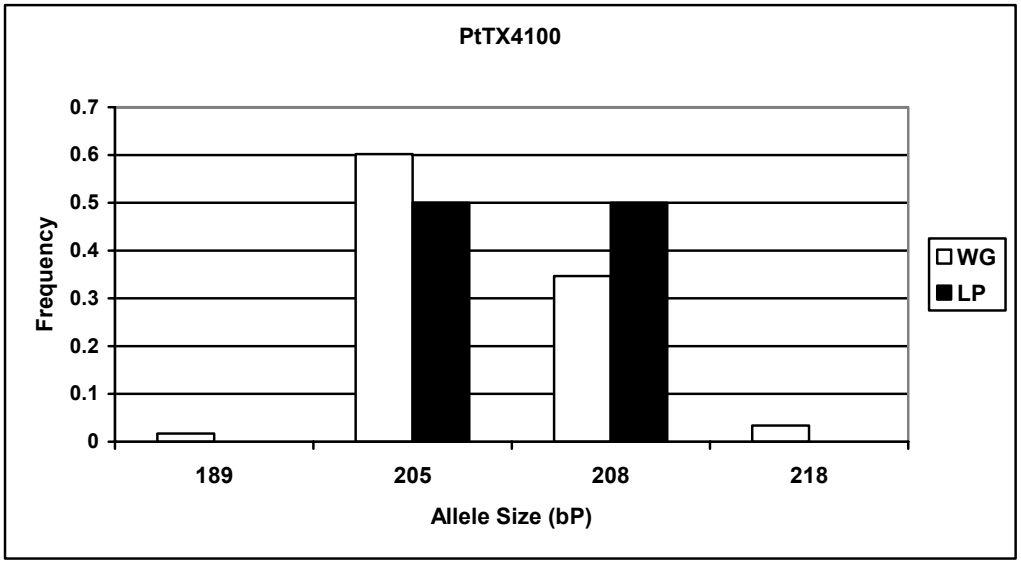
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
342
345
350
358
368
Allele Size (bP)
WG
LP


PtTX4100
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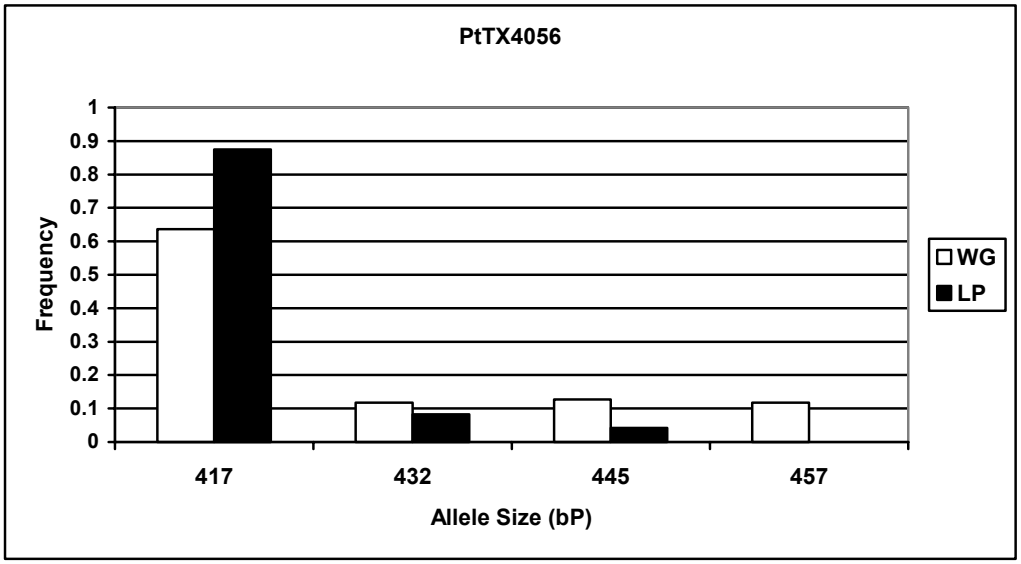
0.5
0.4
0.3
0.2
0.1
0
Frequency
189
205
208
218
Allele Size (bP)
WG
LP


PtTX4056
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0.8
0.7
0.6
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0.4
0.3
0.2
0.1
0
Frequency
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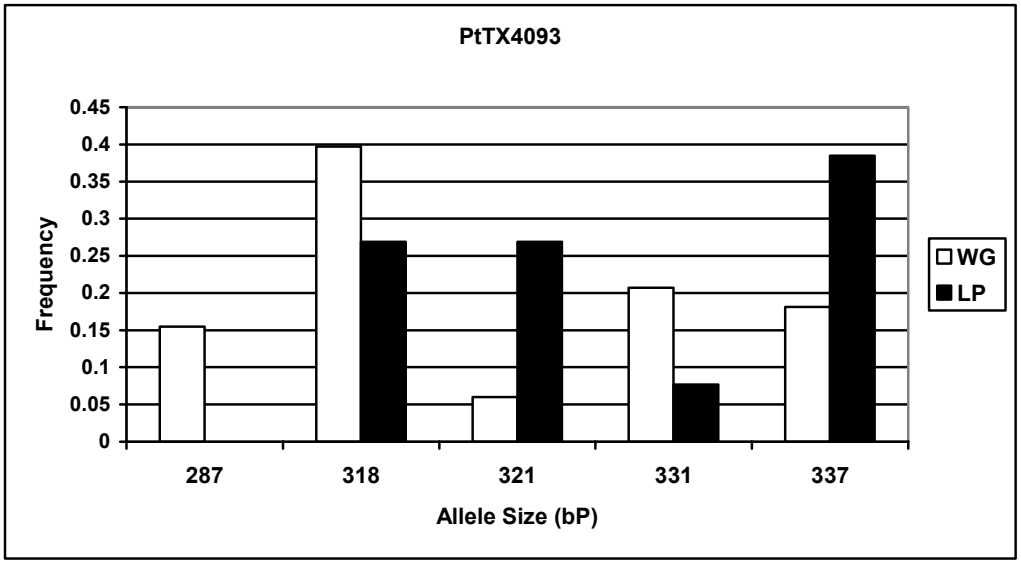
432
445
457
Allele Size (bP)
WG
LP


PtTX4093
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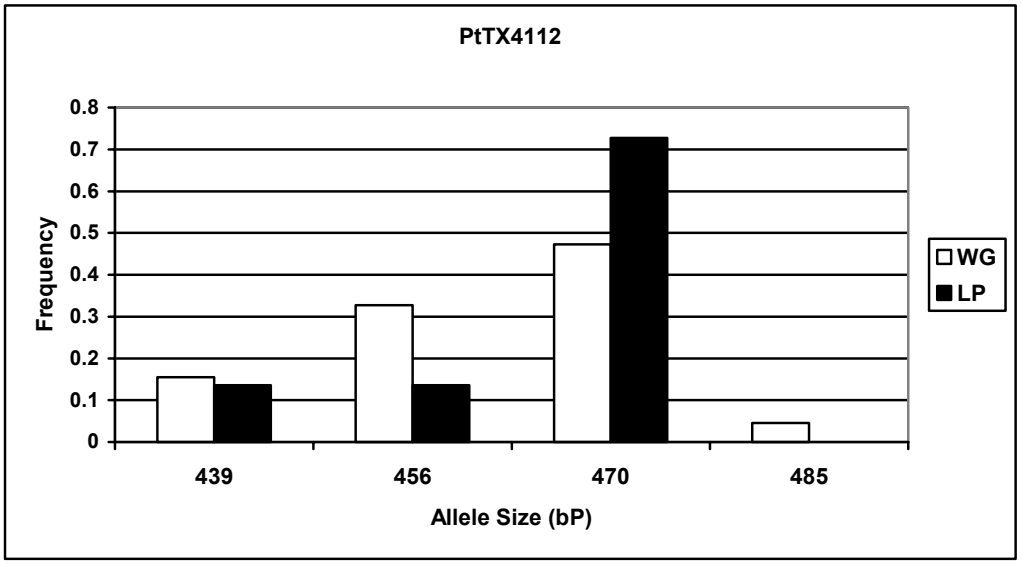
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Frequency
287
318
321
331
337
Allele Size (bP)
WG
LP


PtTX4112
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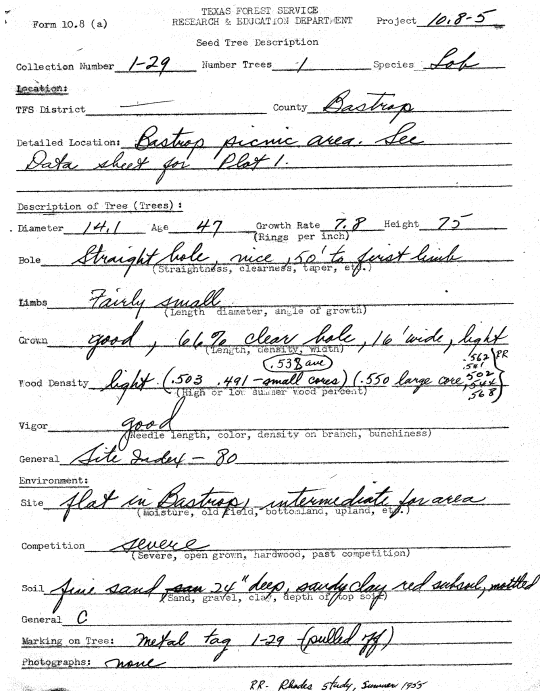
0.6
0.5
0.4
0.3
0.2
0.1
0
Frequency
439
456
470
485
Allele Size (bP)
WG
LP

APPENDIX C

THE ORIGINAL REPORTS AND PHOTOGRAPHS FOR SOME OF THE LOST PINES SAMPLES

Original grade reports for 14 of the 18 Lost Pine samples were obtained from the Western Gulf Forest Tree Improvement Program (WGFTIP). Some of these grade reports show the date when these trees were selected from the wild and grafted into the archive. These reports also show the estimated age of these trees at the time of selection. These reports show that these trees where selected in the early 1950's. These trees where 30-70 years old at the time of selection. This means that these trees date back to 1880's to 1920's. This is important to assure that these trees are of wild origin dating before the domestication of *P. taeda* starting early 1930's. These records are posted as scanned images. Some of these trees are photographed at the time of the selection or later by two to three years. These photos and the information sheet for that photograph will be shown next to it.

The following are scanned images of the original grade sheets for 14 of the 18 Lost Pine samples used in this study. For each tree, there are two records that have been scanned which contained the information. Some records contain only one record.



Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-5

Seed Tree Description

Collection Number 1-29 Number Trees 1 Species Loh

Location:

TFS District — County Bastrop

Detailed Location: Bastrop picnic area. See Data sheet for Plot 1.

Description of Tree (Trees):

Diameter 14.1 Age 47 Growth Rate (Rings per inch) 7.8 Height 75

Bole Straight hole, nice, 50' to first limb (Straightness, clearness, taper, etc.)

Limbs Fairly small (Length, diameter, angle of growth)

Crown good, 66% clear hole, 16' wide, light (Length, density, width)

Wood Density light (.503 .491 - small cores) (.550 large core, .538 ave) (.542 .541 .502 .538 .568) PR (High or low summer wood percent)

Vigor good (Needle length, color, density on branch, bunchiness)

General Site Index - 80

Environment:

Site flat in Bastrop, intermediate for area (Moisture, old field, bottomland, upland, etc.)

Competition severe (Severe, open grown, hardwood, past competition)

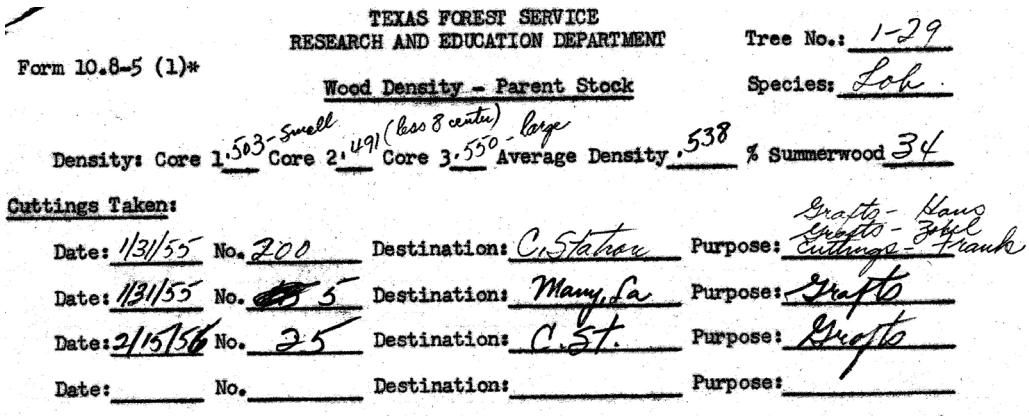
Soil fine sand ~~sand~~ 24" deep, sandy clay red subsoil, mottled (Sand, gravel, clay, depth of top soil)

General C

Marking on Tree: Metal tag 1-29 (pulled off)

Photographs: none

RR. Rhodes Study, Summer 1955



TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (1)* Wood Density - Parent Stock

Tree No.: 1-29

Species: Loh

Density: Core 1 .503 - small Core 2 .491 (less 8 center) Core 3 .550 - large Average Density .538 % Summerwood 34

Cuttings Taken:

Date: 1/31/55 No. 200 Destination: C. Station Purpose: Grafts - Hans Grafts - Zobel Cuttings - Frank

Date: 1/31/55 No. ~~25~~ 5 Destination: Many, La Purpose: Grafts

Date: 2/15/56 No. 25 Destination: C. St. Purpose: Grafts

Date: ____ No. ____ Destination: ____ Purpose: ____

Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-5

Seed Tree Description

4201001
Collection Number 1-50 Number Trees 1 Species Lob.

Location:

TFS District — County Bastrop

Detailed Location: Park, flat picnic grounds. See Plat 1 detailed location

Description of Tree (Trees):

Diameter 12.7 Age 46 Growth Rate 8.2 (Rings per inch) Height 80

Bole Straight, clear 55 feet
(Straightness, clearness, taper, etc.)

Limbs Ave lengths, sweeps up
(Length diameter, angle of growth)

Crown 66 To clear hole, Crown 14' wide
(Length, density, width)

Wood Density .663 (small) .636 (large) (.636 ave) .627 .646
(High or low summer wood percent)

Vigor vigorous
(Needle length, color, density on branch, bunchiness)

General Nearly superior tree for area

Environment:

Site Flat, intermediate for area, near stream
(Moisture, old field, bottomland, upland, etc.)

Competition severe
(Severe, open grown, hardwood, past competition)

Soil fine sand, 24" dep, subsoil red sandy clay, mottled
(Sand, gravel, clay, depth of top soil)

General Site Index 80

Marking on Tree: tag 1-50

Photographs:

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. 1-50

Project No. 1085 Species PT

SUCCESSFUL GRAFTS

Year grafted 1955 Type Bottle No. grafts 14
Stock PT Outplanted Fastrill

Year grafted 1955 Type Bottle No. grafts 1
Stock PC Outplanted Fastrill

Year grafted 1955 Type Dry No. grafts 1
Stock PSw Outplanted Fastrill

Year grafted 1956 Type Dry No. grafts 1
Stock PT Outplanted Fastrill

Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number BAl-1 Number Trees 1 Species Loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: Near creek, in usused roadside park, highway between Bastrop and College Station, E. extension of pine in Bastrop.

Description of Tree (Trees):

Diameter 13" Age 33 Growth Rate 5.4 (Rings per inch) Height 60'

Bole Clear 25', leaning, not well pruned (Straightness, clearness, taper, etc.)

Limbs Rather long, large (Length diameter, angle of growth)

Crown Larger than wished but best in vicinity with semi-straight bole. (Length, density, width)

Wood Density .664 (small core) (High or low summer wood percent)

Vigor Good (Needle length, color, density on branch, bunchiness)

General

Environment:

Site Deep sand, above creek, moderately moist (Moisture, old field, bottomland, upland, etc.)

Competition Severe (Severe, open grown, hardwood, past competition)

Soil Deep sand (Sand, gravel, clay, depth of top soil)

General

Marking on Tree: BAl-1

Photographs:

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BAI-1

Project No. 10.89 Species Lob.

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 1
Stock Lob-Jes Outplanted Fastnill

Year grafted 1955 Type Cleft No. grafts 1
Stock Sonderegger Outplanted Fastnill

Year grafted 55 Type Bot No. grafts 4
Stock PT Outplanted Fast.

Year grafted 1955 Type Dry No. grafts 1
Stock PT Outplanted Fast.

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT
Project 10.89

Seed Tree Description
Date 1954

Collection Number BA1-2 Number Trees 1 Species Lob.

Location:

TFS District — County Bastrop

Detailed Location: On flat near BA1-1. See BA1-1

Description of Tree (Trees):

Diameter Age Growth Rate (Rings per inch) Height

Bole (Straightness, clearness, taper, etc.)

Limbs (Length, diameter, angle of growth)

Crown (Length, density, width)

Wood Density 6 (High or low summer wood percent)

Vigor (Needle length, color, density on branch, bunchiness)

General

Environment:

Site (Moisture, old field, bottomland, upland, etc.)

Competition (Severe, open grown, hardwood, past competition)

Soil (Sand, gravel, clay, depth of top soil)

General

Marking on Tree:

Photographs

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA1-2

Project No. 10.89 Species Lob.

SUCCESSFUL GRAFTS

Year grafted 1955 Type Dry No. grafts 1
Stock Slash Outplanted Fastnet

Year grafted 55 Type Bottle No. grafts 1
Stock PC Outplanted Fast.

Year grafted 55 Type Dry No. grafts 3
Stock PT Outplanted Fast

Year grafted 55 Type Bot No. grafts 5 (5)
Stock PT Outplanted Fast

Year grafted 56 Type Dry No. grafts 1
Stock PT Outplanted Fastnet

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number BA2-1 Number Trees 1 Species Loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: Near tank, State Park, same tree as Ba2PT-51. Tree used for crossing 1952, 1953 and 1954.

Description of Tree (Trees):

Diameter 11.0" Age 45 Growth Rate 10.4 (Rings per inch) Height 5.0

Bole Clear 30', straight (Straightness, clearness, taper, etc.)

Limbs Small, flat angle (Length diameter, angle of growth)

Crown Small, especially good for area (Length, density, width)

Wood Density .616 (small core) (High or low summer wood percent)

Vigor Fair (Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Extremely dry, gravelly hillside (Moisture, old field, bottomland, upland, etc.)

Competition Fair (Severe, open grown, hardwood, past competition)

Soil Gravel (Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: BA2PT-51 yellow paint, seed tree sign

Photographs: Several

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA2-1

Project No. 10.89 Species Lob.

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 1
Stock Lob-Tex. Outplanted Fastnull

Year grafted 1954 Type Cleft No. grafts 1
Stock Lob-Tex Outplanted Fastnull

Year grafted 1956 Type Dry No. grafts 2
Stock PT Outplanted Fastnull

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number BA3-1 (D) Number Trees 1 Species loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: Same as project 10.8-5 tree D. In Bastrop park, picnic area, near creek

Description of Tree (Trees):

Diameter 17.5 " Age 41 Growth Rate 6.2 (Rings per inch) Height 75'

Bole Not straight, some sweep, clear 40 feet
(Straightness, clearness, taper, etc.)

Limbs Somewhat long, relatively large, flat angle
(Length diameter, angle of growth)

Crown Not too long, not dense, rather wide
(Length, density, width)

Wood Density dense .655 (small core)
(High or low summer wood percent)

Vigor Fair
(Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Moist, near creek
(Moisture, old field, bottomland, upland, etc.)

Competition Severe
(Severe, open grown, hardwood, past competition)

Soil Samd
(Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: D, Seed tree sign

Photographs: _____

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA3-1

Project No. _____ Species _____

SUCCESSFUL GRAFTS

Year grafted 1955 Type Bottle No. grafts 3
Stock PT Outplanted Tosh.

Year grafted 1955 Type Dry No. grafts 3 (5)
Stock Sonderegger Outplanted Tastrill

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA3R

Project No. 10.89 Species Loh

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 2
Stock Loh - Tex Outplanted Fastrill

Year grafted 1954 Type Cleft No. grafts 1
Stock Loh - Tex Outplanted Fastrill

Year grafted 1955 Type Bt. No. grafts 7
Stock PT Outplanted Fast.

Year grafted 55 Type Dry No. grafts 13
Stock PT Outplanted Fast.

Year grafted 55 Type cleft No. grafts 13
Stock PT Outplanted Fast

Year grafted 55 Type Bot No. grafts 2
Stock PC Outplanted Fast

Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number BA5-1 Number Trees 1 Species loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: On highway from Bastrop to College Station, several miles E of Bastrop, very nice stand in swale of road, on S. side of road, on Highway right-of-way.

Description of Tree (Trees):

Diameter 17" Age 70 Growth Rate 8.7 (Rings per inch) Height 70'

Bole Straight, clear 45'
(Straightness, clearness, taper, etc.)

Limbs Moderate length, flat angle
(Length diameter, angle of growth)

Crown Moderate for tree size, flat top, broken out
(Length, density, width)

Wood Density .613 small core
(High or low summer wood percent)

Vigor Gair
(Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Swale, moister than around
(Moisture, old field, bottomland, upland, etc.)

Competition Fair, now road on 1 side
(Severe, open grown, hardwood, past competition)

Soil Sandy, deep
(Sand, gravel, clay, depth of top soil)

General Used for collection of BA5PT-53

Marking on Tree: BA5-1, also BA5

Photographs: Yes

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA5-1

Project No. 10.89 Species Lob

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 1
Stock Lob-Tex Outplanted Fastnill

Year grafted 1955 Type Bottle No. grafts 3
Stock Lob-Tex Outplanted Fastnill

Year grafted 1955 Type Dry No. grafts 1 (5)
Stock P. Sou Outplanted Fastnill

Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-7

Seed Tree Description

Collection Number BA5-2 Number Trees 1 Species loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: Same as BA5-1, across road on highway right of way

Description of Tree (Trees):

Diameter 19" Age 69 Growth Rate 7.8 (Rings per inch) Height 70'

Bole Straight, clear for 50', large. (Straightness, clearness, taper, etc.)

Limbs Larger than BA5-1, sharp (Length diameter, angle of growth)

Crown Relatively large, very dense (Length, density, width)

Wood Density .602 (Small core) (High or low summer wood percent)

Vigor Good (Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Good, moister than surrounding (Moisture, old field, bottomland, upland, etc.)

Competition Much (Severe, open grown, hardwood, past competition)

Soil Sand (Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: BA5-2

Photographs: YES

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. BA5-2

Project No. 10.89 Species Lob

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 1
Stock Lob-Jul Outplanted Fastnull

Year grafted 1955 Type Bottle No. grafts 4
Stock Lob-Jul Outplanted Fastnull

Year grafted 55 Type Bot No. grafts 3
Stock P. Sen Outplanted Fastnull

Year grafted 55 Type Dry No. grafts 2 ⑨
Stock P. Sen Outplanted Fastnull

4201002

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH & EDUCATION DEPARTMENT Project 10.8-5

Seed Tree Description

Collection Number E (R-2) Number Trees 1 Species Loblolly

Location:

TFS District _____ County Bastrop

Detailed Location: Bastrop State Park, approximately 150 yards below lake dam. On west edge of creek. Can be seen by cleared "road" from blacktop.

Description of Tree (Trees):

Diameter 18" Age 48 Growth Rate 6.0 (Rings per inch) Height 70-80

Bole Some sweep, leans a little over creek (Straightness, clearness, taper, etc.)

Limbs Fair size, sharp angle, sparse (Length diameter, angle of growth)

Crown Open, few limbs, widely spaced (Length, density, width)

Wood Density Dense .661, .672, .704 .644 ave .625 large core .628 (High or low summer wood percent)

Vigor Fair (Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Moist, along creek (Moisture, old field, bottomland, upland, etc.)

Competition Not heavy too (Severe, open grown, hardwood, past competition)

Soil Sand (Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: Seed tree sign

Photographs: _____

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. E

Project No. 10.8-5 Species PT

SUCCESSFUL GRAFTS

Year grafted 1955 Type Bottle No. grafts 2 (2)

Stock PT Outplanted Fastrill

Year grafted 1955 Type Dry No. grafts 2

Stock PT Outplanted Fastrill

Form 10.8 (a) TEXAS FOREST SERVICE RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number FA1-1 Number Trees 1 Species loblolly

Location:

TFS District _____ County Fayette

Detailed Location: Location as FA1PT on project 10.8-3, on Kunze's land, Warda, on creek flat.

Description of Tree (Trees):

Diameter 16 est. Age 56 Growth Rate 7.7 (Rings per inch) Height 70'

Bole 40' to crown, straight (Straightness, clearness, taper, etc.)

Limbs some rather long, some "feather" limbs on trunk, flat angle (Length diameter, angle of growth)

Crown not particularly narrow (Length, density, width)

Wood Density .589 (small core) (High or low summer wood percent)

Vigor Excellent (Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site On creek, relatively moist in dry area (Moisture, old field, bottomland, upland, etc.)

Competition Rather severe (Severe, open grown, hardwood, past competition)

Soil Gravelly sand (Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: FA1-1 metal tag.

Photographs: _____

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. FA1-1

Project No. 10.89 Species Lob

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 1
Stock Lob-Lob Outplanted ~~Hp~~ Pastrill

Year grafted 1955 Type Dug No. grafts 1
Stock PT Outplanted Pastrill

Year grafted 1955 Type Bottle No. grafts 1
Stock PT Outplanted Pastrill

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH & EDUCATION DEPARTMENT Project 10.8-4

Seed Tree Description

Collection Number FAl-2 Number Trees 1 Species loblolly

Location:

TFS District _____ County Fayette

Detailed Location: On Kunze's land, near Warda, tree same as described for FAlPT in project 10.8-3. Used in 1954 for hybridizing.

Description of Tree (Trees):

Diameter 15" Age 51 Growth Rate 5.7 (Rings per inch) Height 60'

Bole 25' to crown, straight (Straightness, clearness, taper, etc.)

Limbs Medium diameter, rather long, sharp angle. (Length diameter, angle of growth)

Crown Not too good (Length, density, width)

Wood Density .692 (small core) (High or low summer wood percent)

Vigor Good (Needle length, color, density on branch, bunchiness)

General Constant seeder

Environment:

Site Above creek, little drier than FAl-1 (Moisture, old field, bottomland, upland, etc.)

Competition Little (Severe, open grown, hardwood, past competition)

Soil Dry sand and gravel (Sand, gravel, clay, depth of top soil)

General

Marking on Tree: Tag FAlPT-51, yellow spot

Photographs:

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. FAl-2

Project No. 10.751-1 Species PT

SUCCESSFUL GRAFTS

Year grafted 1956 Type Dug No. grafts 5
Stock P. Sand Outplanted Fastrill

Year grafted 1956 Type Dug No. grafts 16
Stock PT Outplanted Fastrill

Year grafted 1956 Type Dug No. grafts 1
Stock PC Outplanted Fastrill

Year grafted 1955 Type Bottle No. grafts 1
Stock PT Outplanted Fastrill

Form 10.8 (a) TEXAS FOREST SERVICE
RESEARCH & EDUCATION DEPARTMENT Project 10.8-9

Seed Tree Description

Collection Number FA2-2 Number Trees 1 Species Loblolly

Location:

TFS District _____ County Fayette

Detailed Location: Same location as FA2 for project 10.8-3. Near Warda, roadside park.

Description of Tree (Trees):

Diameter app. 11 in. Age 59 Growth Rate 11 (Rings per inch) Height 60±

Bole 40' to main crown (Straightness, clearness, taper, etc.)

Limbs Small, sharp angle (Length diameter, angle of growth)

Crown Small width (Length, density, width)

Wood Density .579 (small core) (High or low summer wood percent)

Vigor Good, nice sharp top (Needle length, color, density on branch, bunchiness)

General _____

Environment:

Site Dry upland. (Moisture, old field, bottomland, upland, etc.)

Competition Severe (Severe, open grown, hardwood, past competition)

Soil Gravel-sand hill, very dry (Sand, gravel, clay, depth of top soil)

General _____

Marking on Tree: FA2-2

Photographs: _____

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3) Tree No. FA2-2

Project No. 10.89 Species Lob.

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 2 1
Stock Lob-Ind Outplanted Fastvill

Year grafted 1955 Type Bottle No. grafts 24 11
Stock Lob-Ind Outplanted Fastvill

Year grafted 1955 Type Dry No. grafts 14 18
Stock Lob-Ind Outplanted Fastvill

Year grafted 1955 Type Bottle No. grafts 1
Stock P. Sen Outplanted Fastvill

Year grafted 1955 Type Dry No. grafts 1
Stock P. Sen. Outplanted Fastvill

TEXAS FOREST SERVICE
RESEARCH AND EDUCATION DEPARTMENT

Form 10.8-5 (3)

Tree No. FA2F

Project No. 10.89

Species Loblolly

SUCCESSFUL GRAFTS

Year grafted 1954 Type Bottle No. grafts 4

Stock Lob-Lel Outplanted Fastrill

Year grafted 1955 Type Dry No. grafts 1

Stock PT Outplanted Fastrill

The following are scanned photographs of some of the original trees from the Lost Pine samples that have been used in this study. The scanned information cards are shown next to each photo.

10.722-1
Picture No. Roll No. Date: 3-1954
Taken by: Ray E. Goddard
Location: Bastrop Co.
Subject: Drought Hardy
Description: 22 & 23 Tree BA1-1
Negatives--Roll 3 Nos. 9 & 10

10.722-1

Picture No. **Roll No.** **Date:** 10-55

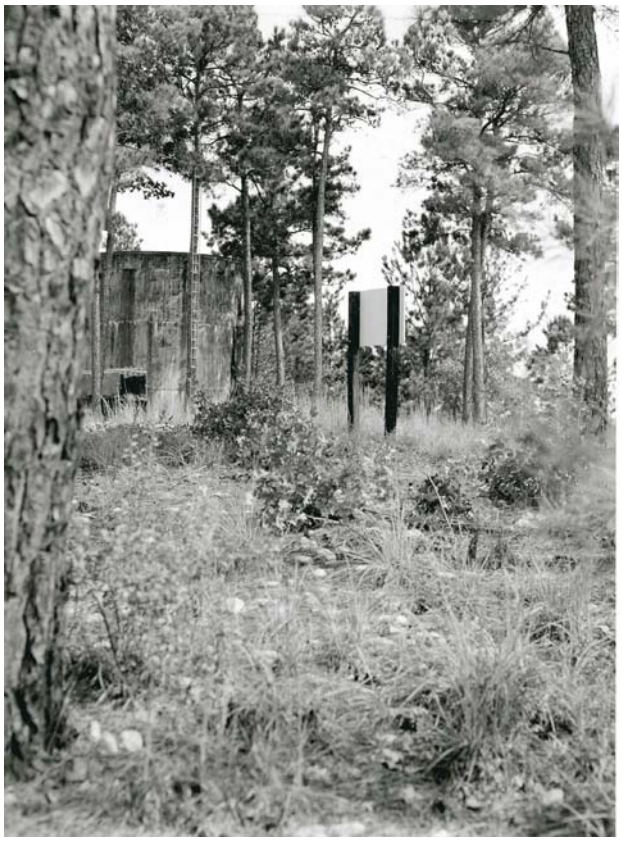
Taken by: Cech

Location: Bastrop State Park

Subject: Tree BA2-1 (PT1)

Description: 148,149 Tree BA2-1 (PT1) with ladder.

Neg. 10.722-1 95,96



10.722-1

Picture No. Roll No. Date: 10-55

Taken by: Cech

Location: Bastrop State Park

Subject: Tree BA2-1 (PT1)

Description: Tree BA2-1 (PT1) with ladder.

Neg. 10.722-1 95-96.

10.722-1
Picture No. Roll No. Date: 10/55

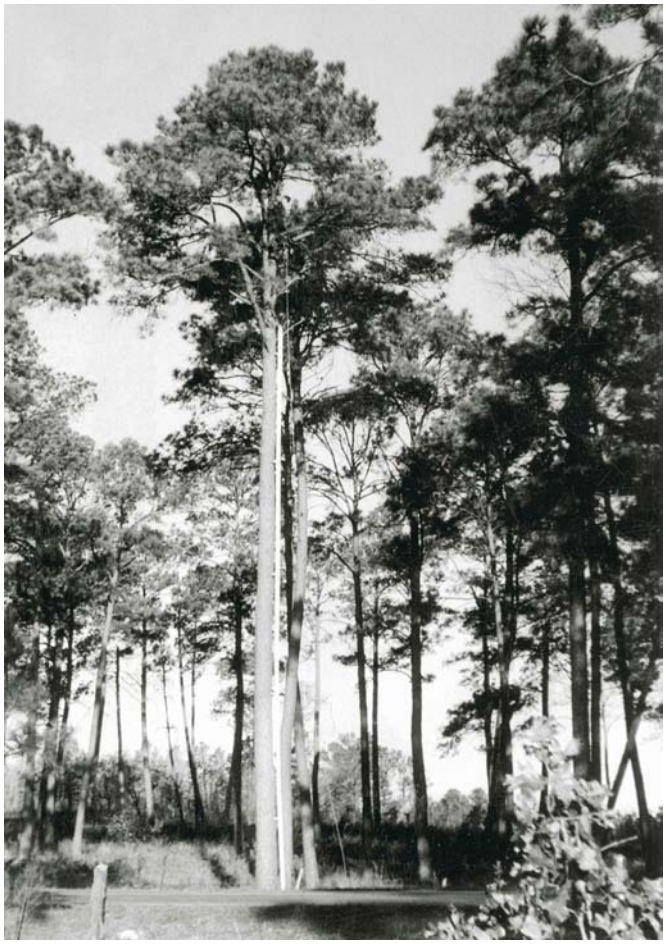
Taken by: Zobel

Location: Bastrop State Park, picnic area.

Subject: BA3 collection

Description: Cech climbing trees for BA3 collection.

Negatives: 74, 75



10.722-1

Picture No. Roll No. Date: 4-1954

Taken by: B. Zobel

Location: Bastrop Co.

Subject: Seed Orchard.

Description: 67,68. BA5-2 from which scions collected, 1954.

Negatives: Roll #7, nos. 29,30.

10.722-1
Picture No. Roll No. Date: 10/55

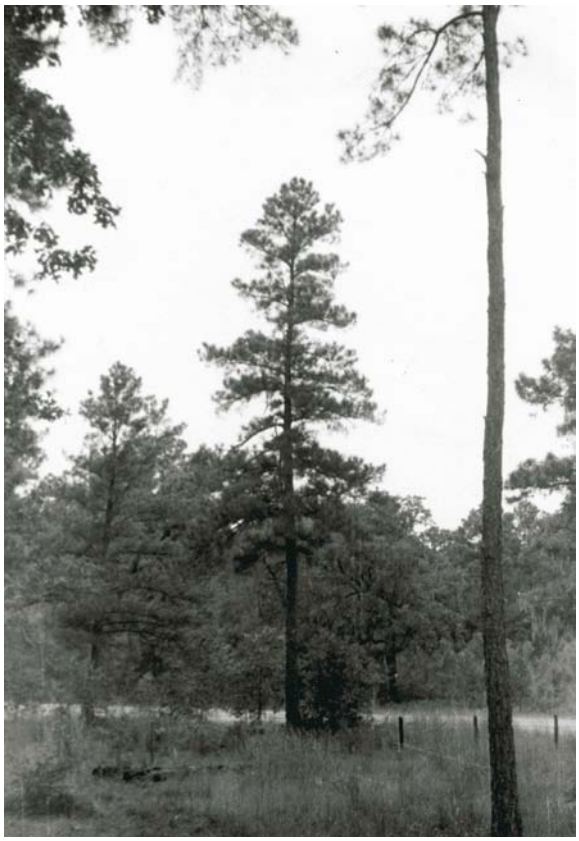
Taken by: Cech

Location: Fayette Co., near Warda

Subject: Gone Colledting.

Description: 157-158 Zobel collecting from PT4 (Fal-2)

Neg. 104-105 (TFS ~~——~~→



Picture No. Roll No. Date: March '54

Taken by: E. Zobel

Location: Fayette Co.

Subject: Drought Resistance

Description: 50. Tree FA2

Negative: Roll 6 no. 36

Picture No. Roll No. Date: 4-1954

Taken by: B. Zobel

Location: Bastrop Co. & Fayette Co.

Subject: Seed Orchard

Description: 62. Tree FA3-1 from which scions collected, 1954.
63,64. Tree BA3-1 from which scions collected, 1954.
65. Tree BA2~~1~~1 from which scions collected, 1954
66. Tree BA5~~1~~1 from which scions collected, 1954.

Negatives: Roll #7, Nos. 22-28.
(22 and 23 alike).

General

Picture No. Roll No. Date: 10/55

Taken by: Zobel

Location: Bastrop State Park, picnic area.

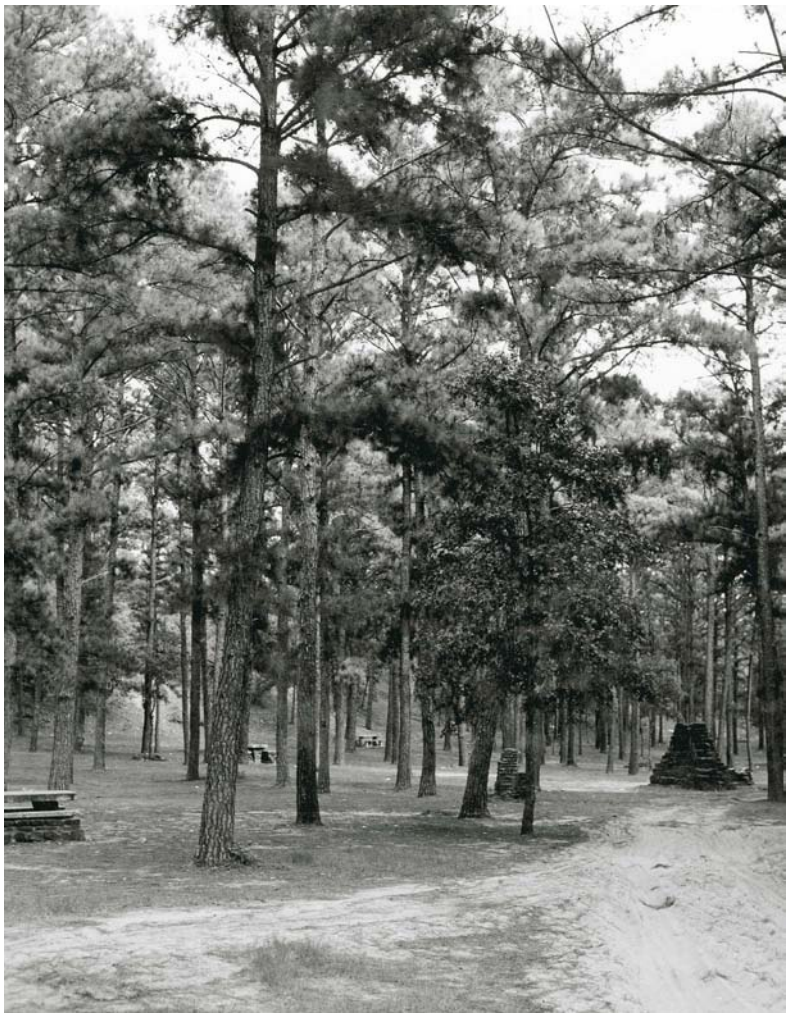
Subject: Cone collection

Description: 57,58 Equipment and camp used during cone collection.

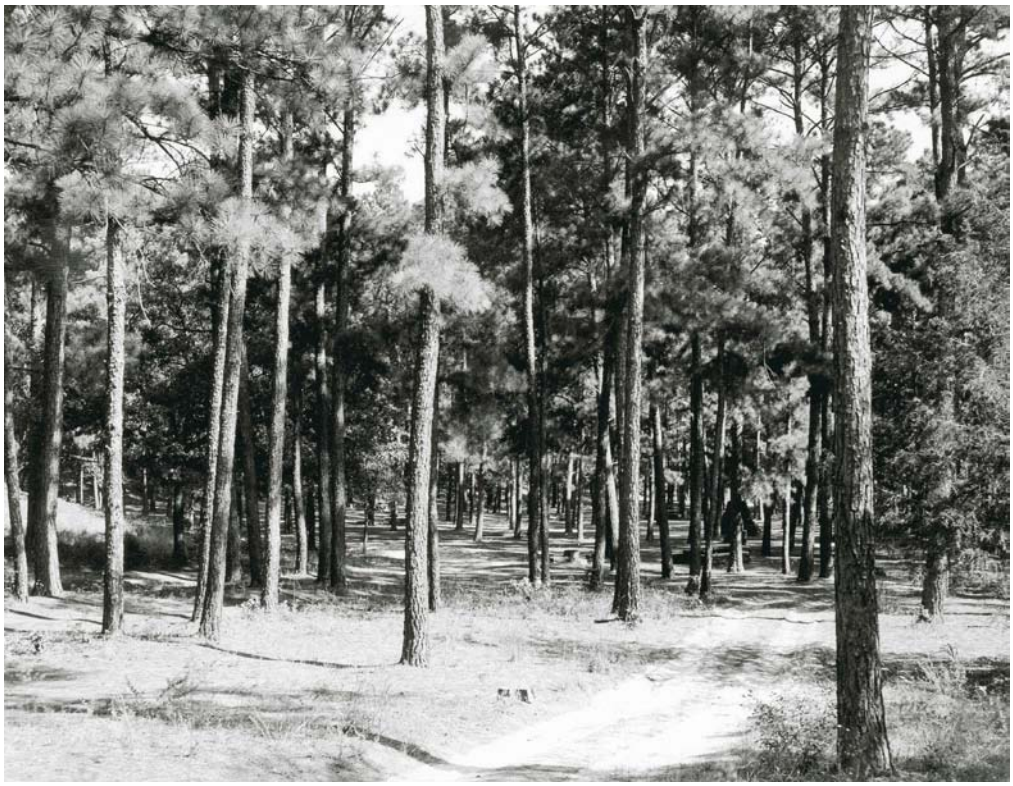
Negatives: 29, 30

The following are scanned photographs from the picnic area in the Lost Pines of Bastrop State Park. Photographs are designated by the code from 61a to 61f. The scanned information card on these photos is shown at the end.

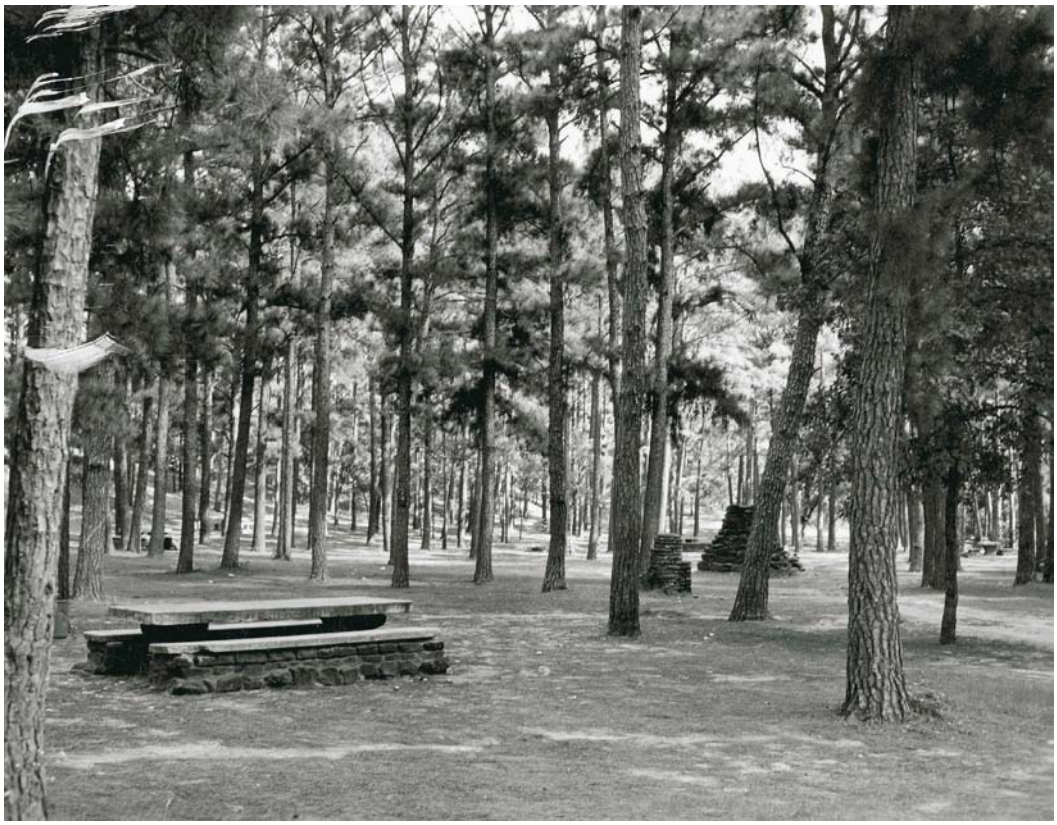
61a.



61b.



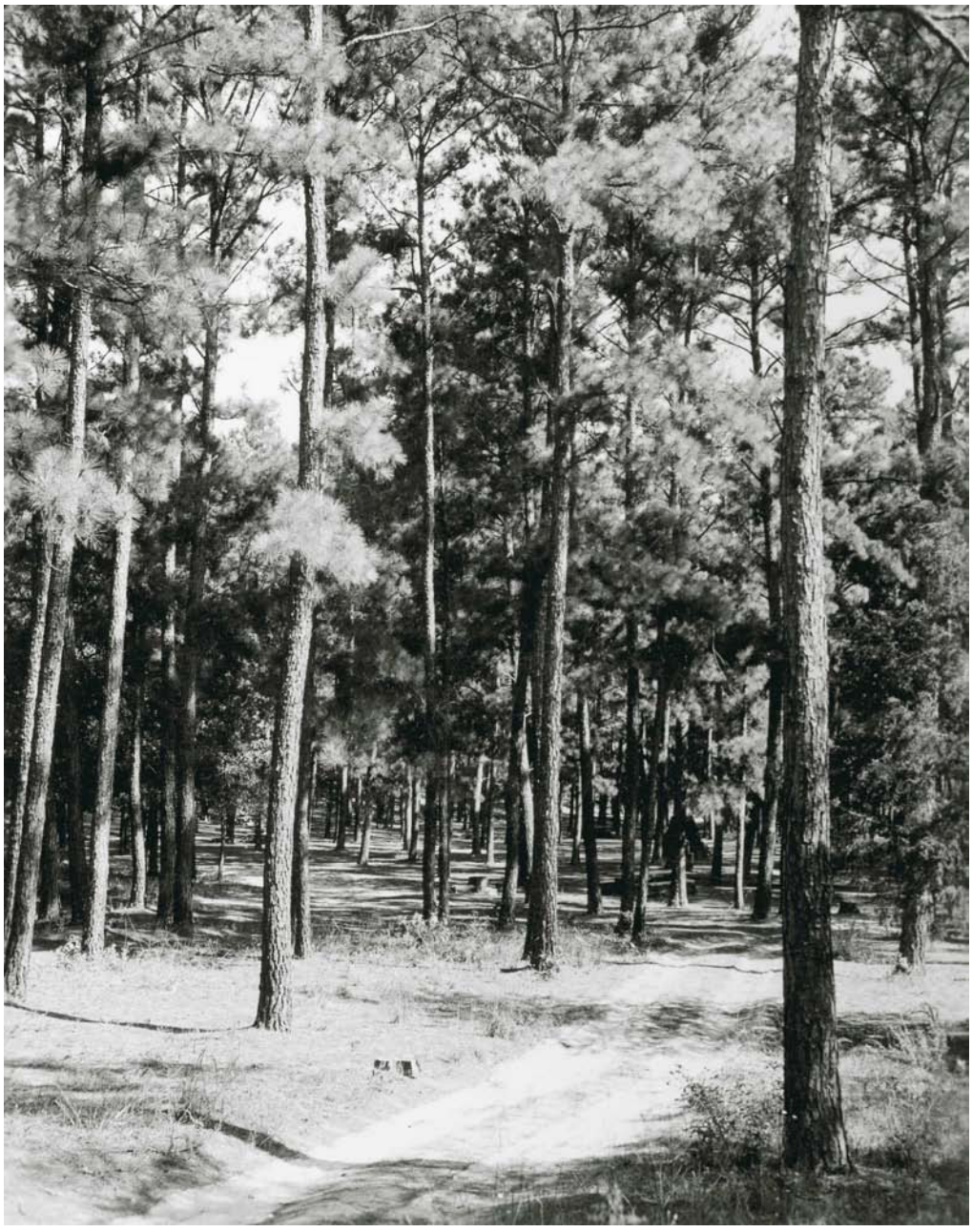
61c.



61d.

61e.

61f.



Information sheet for 61a-61f.

~~[illegible]~~ Drought Resistance

Research

File designation ~~FOREST REGIONS~~

~~Loblolly Type 7.1-A~~ Genetics 6.15

Description 61, a,b,c,d,e,f - Are all scenes of loblolly pine trees taken at the Bastrop State Park; several showing the picnic area.

Location Bastrop State Park, Bastrop

County Bastrop

APPENDIX D

Table D.1

Results of the G-test show that soils inside Bastrop State Park and Buescher State Park are not a representative sample of the soils in Bastrop County.

Soil variable	G-statistics	Degrees of freedom (df)	P-level
Drainage	33.33	2	<0.001
Surface texture	110.7	7	<0.001
Slope	61.13	5	<0.001
Permeability	16.90	3	<0.001
Clay (all layers)	21.98	4	<0.001
Clay (layer 1)	16.64	3	<0.001
Clay (layer 2)	4.65	1	<0.05
Clay (layer 3)	22.30	2	<0.001
Organic matter	42.34	5	<0.001
Bulk density	3.41	1	ns (>0.05)
Soil reaction (pH)	31.72	7	<0.001
Cation exchange capacity (CEC)	24.87	3	<0.001

Table D.2

Results of the G-test show that soils where the pine grew inside the two state parks in 1949 are not significantly different from the soils where the pine grew in 1995 inside the state parks.

Soil variable	G-statistics	Degrees of freedom (df)	P-value
Drainage	0.108	2	ns (>0.1)
Slope	0.92	6	ns (>0.1)
Surface texture	3.37	7	ns (>0.1)
Cation exchange capacity	4.02	5	ns (>0.1)
Clay (all layers)	3.34	5	ns (>0.1)
Organic matter	1.80	5	ns (>0.1)
Permeability	3.52	4	ns (>0.1)
Soil reaction (pH)	2.5	8	ns (>0.1)
Bulk density	1.1	1	ns (>0.1)

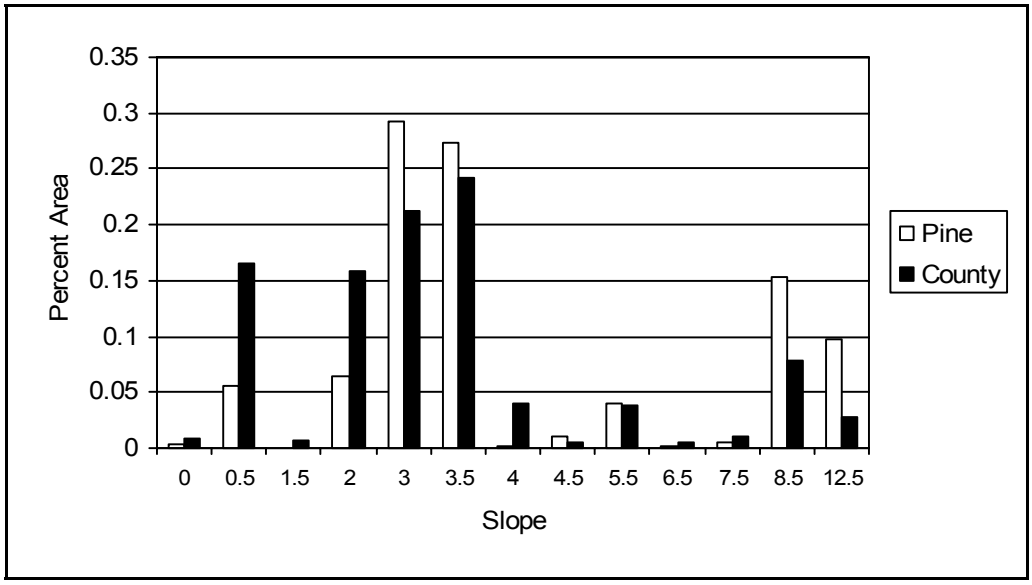


Fig. D.1. Percent pine area grows on different classes of slopes compared to the percent area for the same classes in the whole county. Pines prefer some classes over others.

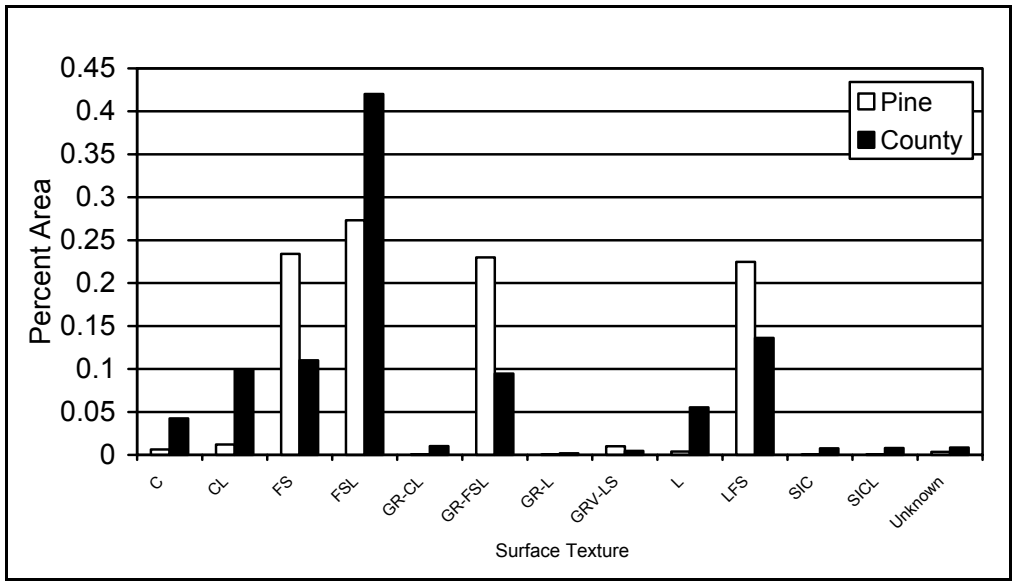


Fig. D.2. Percent pine area grows on different classes of surface texture compared to the percent area for the same classes in the whole county. Pines prefer some classes over others.

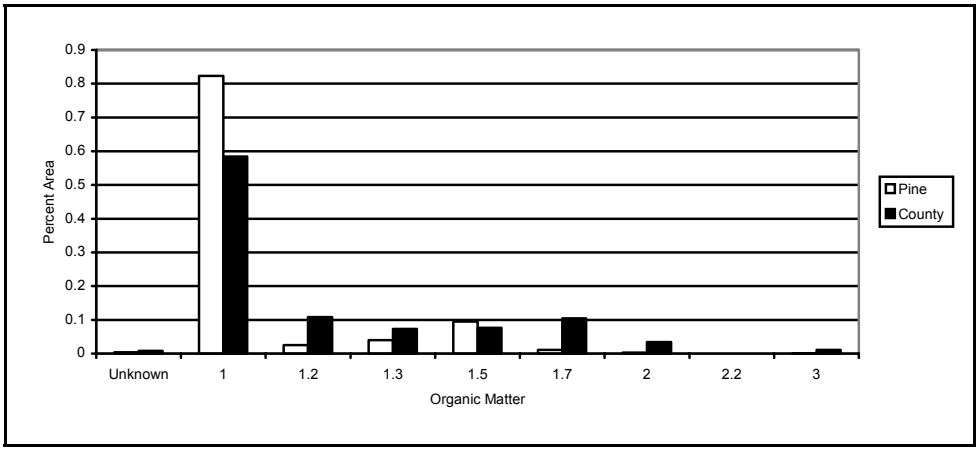


Fig. D.3. Percent pine area grows on different classes of organic matter compared to the percent area for the same classes in the whole county. Pines prefer some classes over others.

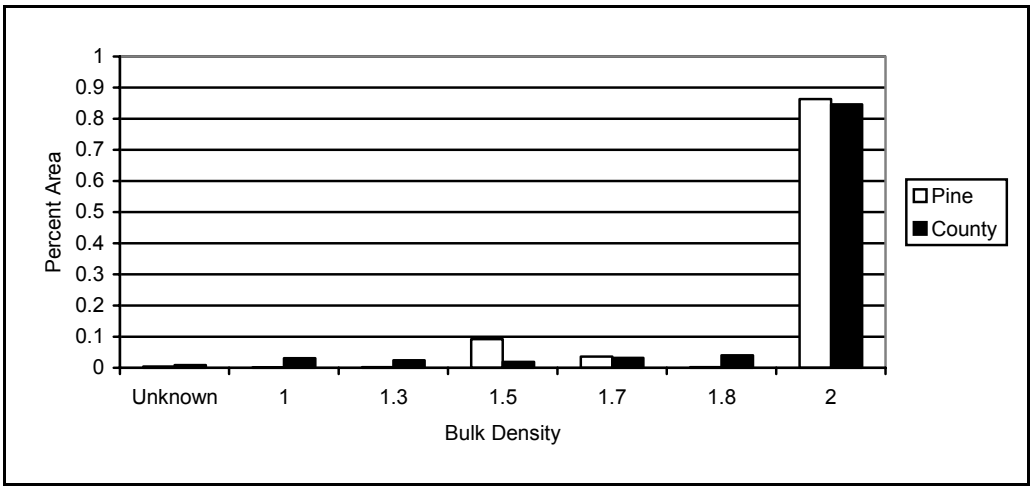


Figure D-4. Percent pine area grows on different classes of bulk density compared to the percent area for the same classes in the whole county. Pines prefer some classes over others.

APPENDIX E

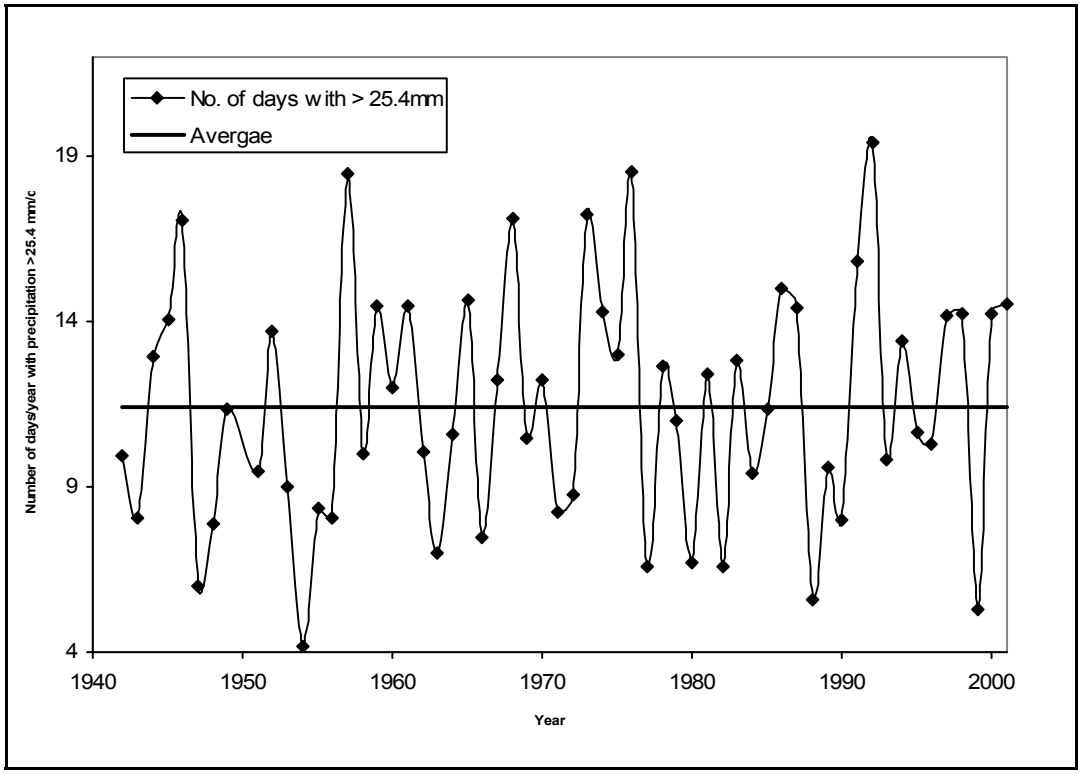


Fig. E.1. Number of days/year with precipitation more than 25.4 mm for Bastrop County. The 60-year average is about 11.4 days which mean at least ¼ of the annual rainfall come in 11.4 days. There is a great fluctuation from one year to the next with arrange from 4.2 to 19.4.

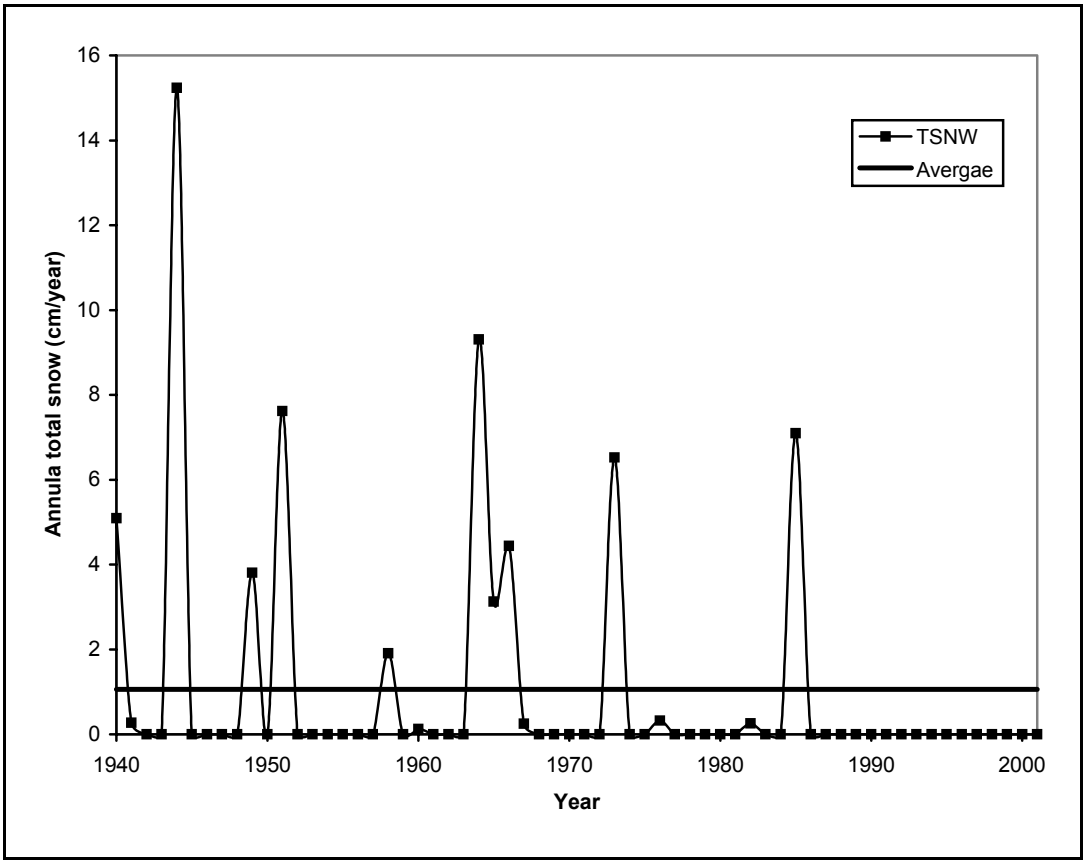


Fig. E.2. Total annual snow (TSNW) in cm for Bastrop County shows that the average is about 1.05 cm. There is a fluctuation from one year to the next with arrange from 0 to 15 cm.

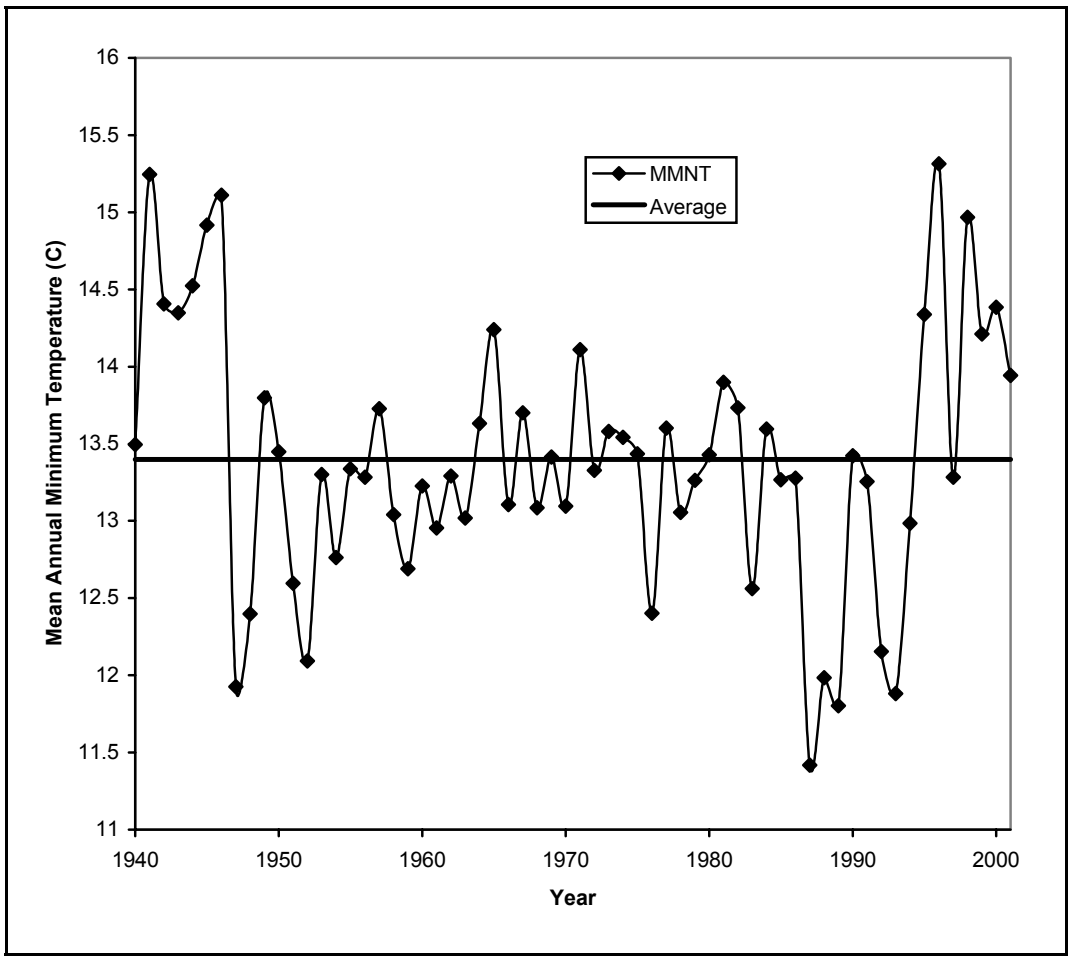


Fig. E.3. Monthly mean minimum temperature (MMNT) for Bastrop County shows that the average is about 13.4 C. Trend is the same as the monthly mean temperature (MNTM).

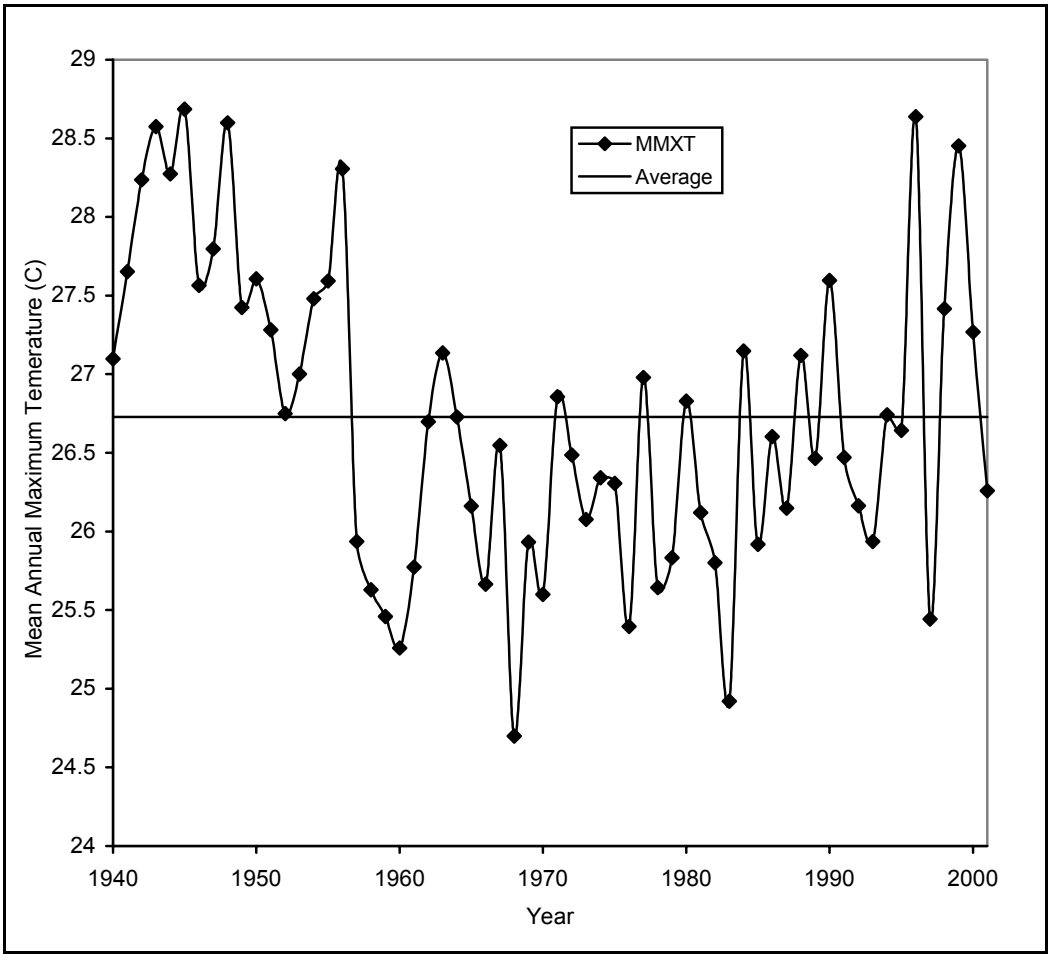


Fig. E.4. Monthly mean maximum temperature (MMXT) for Bastrop County shows that the average is about 26.7 C. Trend is the same as the monthly mean temperature (MNTM).

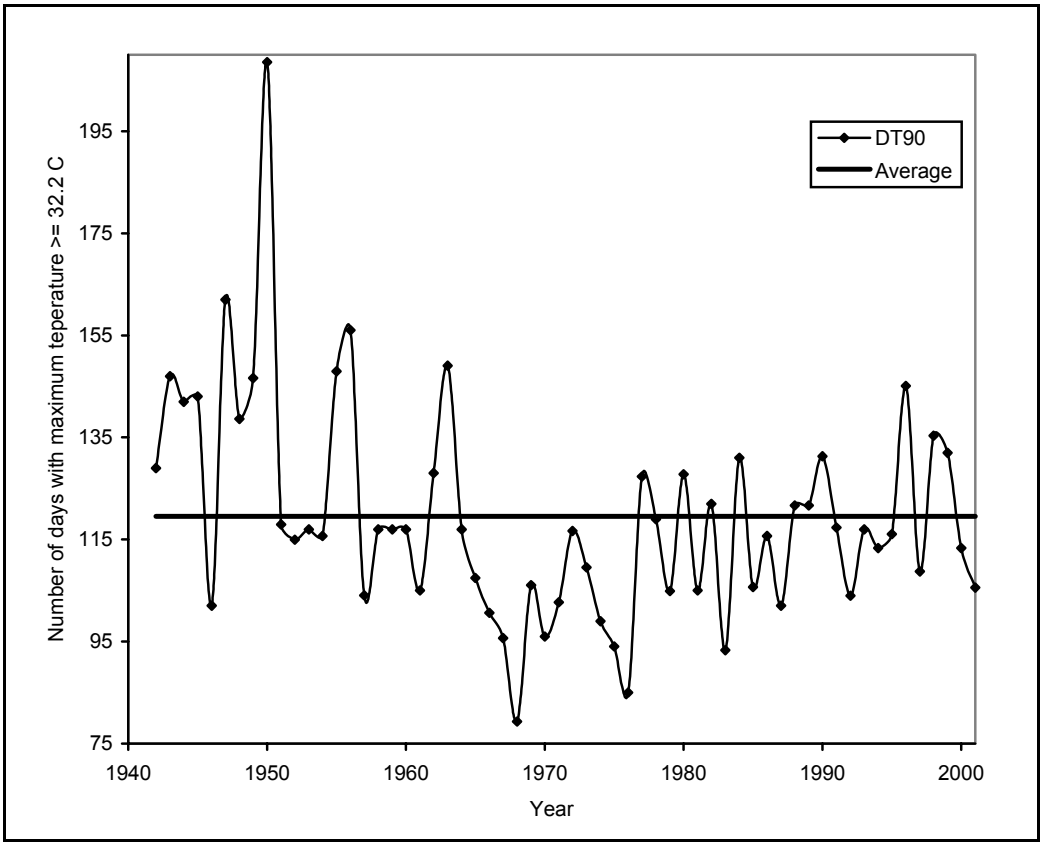


Fig. E.5. Number of days/year with temperatures equal or greater than 32.2 C for Bastrop County. The average is about 120 days. There is a great fluctuation from one year to the next with a range from 79 to 209.

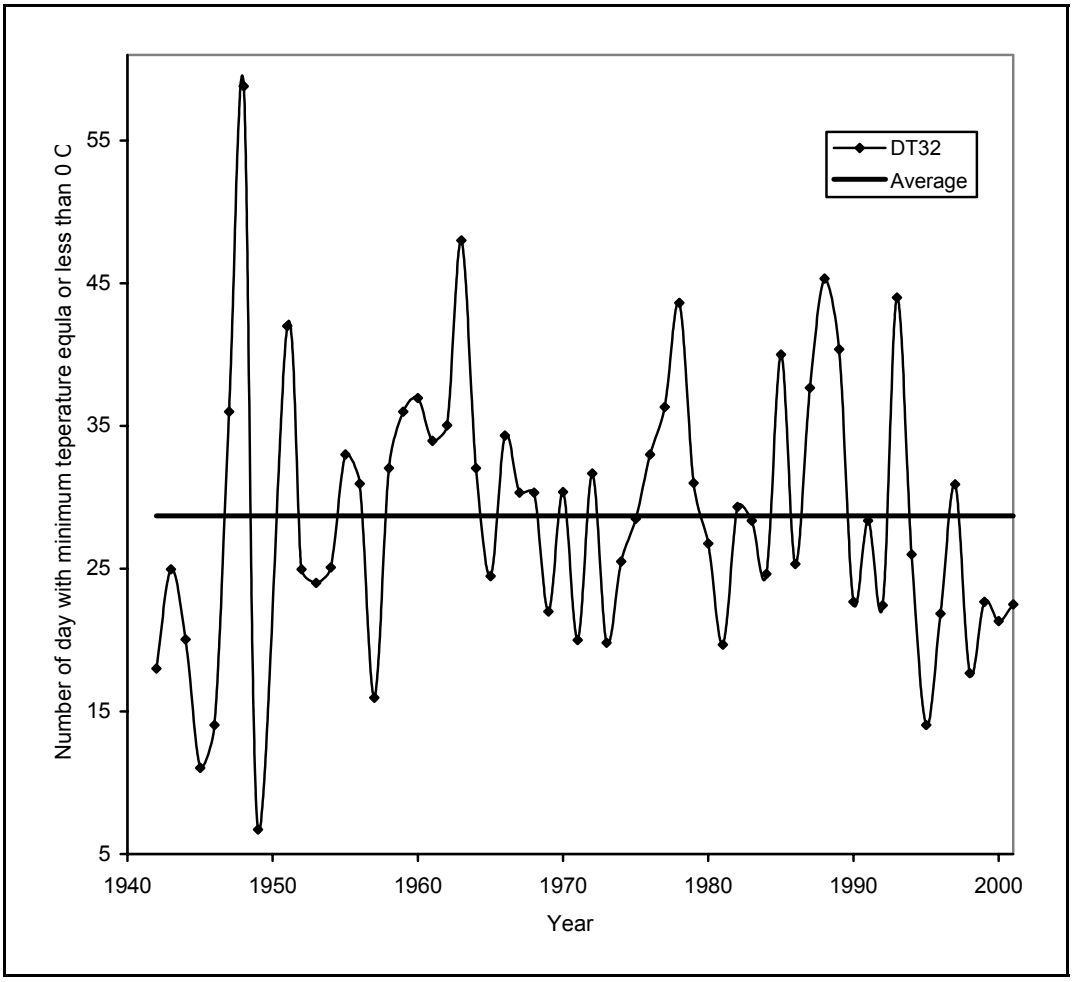


Fig. E.6. Number of days/year with temperatures equal or less than 0 C for Bastrop County. The average is about 29 days. There is a great fluctuation from one year to the next with a range from 7 to 59.

VITA

Mohammad Ali Mustafa Al-Fakir Al-Rababah

Department of Natural Resources & Environment

Faculty of Agriculture, Jordan University of Science & Technology

P.O. Box 3030 Irbid 22110 Jordan

EDUCATION

B.S. Plant Production, June 1993

University of Jordan, Amman, Jordan

M.S. Plant Production/Genetic Resources of Field Crops, August 1996

University of Jordan, Amman, Jordan

Ph.D. Forestry, August 2003

Texas A&M University, College Station, TX 7783

EXPERIENCE

Manager of an Agricultural establishment and a farm 1993-1998.

Teaching Assistant (1995-1996) for a course in field crops at the University of Jordan and (2002-2003) at Texas A&M University for an advanced remote sensing course and basic computer application course.

Research Assistant (1994-1996) at the University of Jordan and (2000-2003) at the Department of Forest Science at Texas A&M University